International Journal of Veterinary and Animal Research 7(3): 75-78, 2024 **RESEARCH ARTICLE**

IJVAR

International Journal of Veterinary and Animal Research

Journal homepage: https://ijvar.org/index.php/ijvar

Investigation of the Antibacterial Effectiveness of Various Licensed Surface Disinfectants

Sibel Kızıl [1✉], Burcu Gürer Giray [2], Şahin Direkel [3], Emine Gül [4], Efsun Melike Çeçen [1], Asya Kazan [1], Gürol Emekdaş [5], Nilüfer Alkan [6]

[1] *Kırıkkale University, Faculty of Veterinary Medicine, Department of Microbiology, Kırıkkale, Türkiye.*
[2] *Yalova University, Faculty of Medicine, Department of Medical Microbiology, Yalova, Türkiye.*
[3] *Malatya Turgut Özal University, Faculty of Medicine, Department of Medical Microbiology, Malatya, Türkiye.*
[4] *Eskişehir Osmangazi University, Department of Industrial Microbiology, Eskişehir, Türkiye.*
[5] *Turkish Blood Foundation, Medical Microbiology, İstanbul, Türkiye.*
[6] *Hacettepe Üniversitesi, English Language and Literature, Faculty of Letters, Ankara, Türkiye.*

ARTICLE INFO

Received: 25/03/2024
Accepted: 11/12/2024

DOI: 10.5281/zenodo.14583329

✉**Corresponding Author:** sibelkizil@kku.edu.tr

Keywords

Antibacterial effectiveness
E. coli
P. aeruginosa
S. aureus
Surface disinfectants

Cite this article as: *Kızıl S, Giray BG, Direkel Ş, Gül E, Çeçen EM, Kazan A, Emekdaş G, Alkan N 2024. Investigation of the Antibacterial Effectiveness of Various Licensed Surface Disinfectants. International Journal of Veterinary and Animal Research, 7(3): 75-78. DOI: 10.5281/zenodo14583329.*

ABSTRACT

The concept of hygiene has become more important today due to pandemic infections. Many products on the market have been licensed, but whose antibacterial effectiveness has not been checked later. For this reason, the study aimed to determine the antibacterial activities of surface disinfectants available in the market for public and personal use, based on the international standard. Eight different surface disinfectants, licensed from the authority and for public and personal use, were collected and processed without any dilution (100%). In the study, standard strains *Staphylococcus aureus* (*S. aureus*) (ATCC 6538), *Escherichia coli* (*E. coli*) (ATCC 10536), and *Pseudomonas aeruginosa* (*P. aeroginosa*) (ATCC 15442) were used. Exposure conditions; it was done for 5 minutes and under clean conditions (0.3 g/L bovine albumin solution) at room temperature (22-24°C). It was determined that the eight surface disinfectants included in the study showed different levels of antibacterial activity. When the logarithm differences and antibacterial activities of surface disinfectants are examined as percentages; while the logarithm difference of three disinfectants against the three bacteria examined was ≥ 5; the logarithm difference of five disinfectants against two bacteria is ≥ 5; the percentage of those effective against all three bacteria was 37.5%; the percentage of those effective against both bacteria was found to be 62.5%. Due to the improprieties detected in the antibacterial activities of the surface disinfectants examined, when their antibacterial activities are examined, although they are licensed, it is seen that the products do not provide the specified antibacterial activity. The selection of disinfectants used to neutralize bacteria that cause infections, the determination of their antibacterial activities, and the sustainability of the determined antibacterial activity are of great importance. For this reason, it is necessary to check the antibacterial activities of disinfectants even after the registration stage and to select disinfectants according to their effectiveness to prevent infections.

INTRODUCTION

Nowadays, the concept of hygiene has become more important due to pandemic infections. Many disinfectants are used individually or institutionally for hygienic purposes. Especially due to the Covid-19 pandemic, the use of disinfectants has become much more common and many disinfectants with different properties have been used.

Chemical disinfection is used on various surfaces due to its wide usage area, cost-effectiveness, and lack of need for mechanical devices. Among the many chemical disinfectants authorized by the competent authorities, those generally used in hospitals and at home, include formaldehyde, glutaraldehyde, hydrogen peroxide, ozone, peracetic acid, phenolics, and quaternary ammonium compounds. Long-term use of disinfectants may cause health risks, human toxicity, and eco-toxicity (Gessi Alessandro, 2023). During the Covid-19 epidemic, there was a shortage of masks and disinfectants. Therefore, instead of alcohol (76.9-81.4% ethanol solution), various disinfectants whose effectiveness was researched and received permission from authorized institutions were used (Kameda et al., 2022).

Aqueous hypochlorous acid (HOCl), which has broad effectiveness against pathogens and is environmentally safe, is actively used. The final product is water and salt; it has no ecotoxicological effects. Hypochlorous is routinely applied for environmental disinfection. HOCl is successful in neutralizing the most resistant infectious agents. HOCl is highly pure, reliable, and has consistent production capability in industrial quantities. It has the feature to meet the needs of pandemics; it is a disinfectant that is affordable in large quantities. HOCl is currently included in the World Health Organization's (WHO) list of biocides effective against coronavirus. In many different brands, aqueous HOCl formulations have been approved for topical use by the Food and Drug Administration (FDA) in the United States of America (USA), again in the European Union (EU) as a Class III medicinal product, and also in Japan by the Ministry of Health (WHO, 2021).

Sodium hypochlorite (SH), usually mixed with a strong alkali, is a disinfectant with a wide antibacterial effect for bacteria and viruses with or without many spores; however, it should not be contacted. High temperatures and ultraviolet light (UV), on the other hand, degrade hypochlorous acid. For these reasons, the effects of reagents are variable and inconsistent. Hypochlorous acid is used as an alternative to alcohol; however, it has a poor shelf life and storage difficulties. In the studies conducted, it is shown that hypochlorous acid is more practical, reliable, and comfortable than alcohol. For the ideal use of hypochlorous acid, it should not be stored and should be used immediately, stored in a cool and dark places (Kameda et al., 2022). With benzalkonium chloride, Choi et al., (2020) conducted a mammalian exposure study, which they did; they found that it causes lung damage even at fairly low levels.

HOCl is the strongest oxidant of the chlorine family, stronger than sodium. It is slightly acidic, at a neutral pH (5-7), and is located in the white blood cells of all mammals. In addition to being cheap, it is water-soluble, non-toxic, and attracts bacteria thanks to its neutral charge and affects the cell wall of bacteria, causing them to die quickly (Practice Guidance for Health Care Environmental Cleaning, 3rd eBook, 2022).

Disinfectants also have many disadvantages. Studies conducted show that the use of SH is responsible for a high rate of poisoning. Sodium hypochlorite accounted for 62.1% of the poisonings during the Covid-19 pandemic, followed by non-alcoholic disinfectants, 36.7%, and hand sanitizers, 36.7%. These products cause indoor air pollution, asthma, and allergies; 0.1% concentration of SH, 70-90% ethanol, or isopropyl alcohol irritates the respiratory tract, eyes, or skin. In addition, SH causes the formation of organic chlorinated compounds, becoming very toxic to humans and the environment. SH droplets that remain in the air for 30 minutes after spraying become quite harmful. Hand sanitizers containing quaternary compounds such as benzalkonium may irritate the skin and respiratory system, triggering asthma. Ozone also has harmful effects on health. It has been determined that its concentration, which is safe for humans, cannot provide adequate disinfection in indoor control. In a study, it was found that when used at a concentration of 0.3 ppm, the time to inactivate 90% of viruses is more than 100 minutes. Nanomaterials have been developed that will reduce some of the negative aspects of chemical disinfectants and differ in terms of harmfulness, abrasiveness, and bacterial resistance. The antibacterial effect of silver and silver nanoparticles has been studied, and it has been found that even safe doses for repeated exposures such as skin, inhalation, or ingestion can lead to health problems (Gessi Alessandro, 2023).

There are many licensed disinfectants on the market. However, the question of the adequacy of the antimicrobial activities of these disinfectants is in doubt. For this reason, the study aimed to determine the antibacterial activities of commercially available surface disinfectants for public and personal space use based on the International Standard determined by the "Biocidal Products Regulation".

MATERIALS AND METHODS

Sampling

In the study, eight different surface disinfectants (Active anionic oxygen natural water, quaternary ammonium, 5% anionic surfactant, didesil dimetil amonyum klorür (120 g/L), Ahp: Accelerated hydrogen peroxide, cationic polymer layer, octadecyl dimethyl ammonium chloride, hydrogen peroxide, deionized water, deodorizing, general surface cleaning liquid) licensed by competent authority, with different active ingredients on the market, were processed without any dilution (100%).

Analysis

S. aureus (ATCC 6538), *E. coli* (ATCC 10536), and *P. aeruginosa* (ATCC 15442) standard strains were used and exposed to disinfectants for 5 minutes. In the study *E. coli* ATCC 10536 was used instead of *Enterococcus hirae* ATCC 10541 specifed in the standard. Exposure conditions was conducted at room temperature (22-24°C) with 0.3 g/L bovine albumin solution under clean conditions. The standard strains were adjusted to be $1.5x10^8$-$5x10^8$cfu/mL with test suspension (N) diluent. One mL of bacterial suspension was added to the tube containing 1 mL of inhibitory substance (0.3 g/L bovine albumin solution). It was mixed and waited for 2 minutes. 8 mL of disinfectant to be tested (100%) was added to it. After mixing, it was waited for 5 minutes. From here, 1 mL of sample was taken and transferred to a tube with 8 mL of neutralizer (Bovine serum albumin) and 1 mL of sterile distilled water. It was mixed and waited for 5 minutes. Then, 1 mL of the neutralized mixture was taken and was inoculated to 2 Tripticase Soy Agar (TSA). Plates were incubated at 36±1°C for 24 hours. At the same time, for experimental control: validation suspension was prepared, experimental conditions were checked, neutralizer control was performed, and dilution-neutralization validation was determined. The obtained results, titer calculation, and logarithm reduction were calculated as specified in the relevant standard (BS EN 13727: 2012+A2: 2015).

RESULTS

The eight disinfectants included in the study were tested under clean conditions without any dilution and were bactericidal at different levels within 5 minutes against *S. aureus*, *P. aeruginosa*, and *E. coli*, which are the microorganisms that must be used in the effectiveness test of standard disinfectants and antiseptics were found to have an effect. The logarithm differences of the disinfectants (those greater than and equal to ≥ 5) were determined; their bactericidal activity against the tested standard strains was determined.

In our study, active anionic oxygen natural water is 100% effective against *E. coli* and *P. aeruginosa*, 98.875% against *S. aureus*; quaternary qmmonium is 100% effective against *E. coli* and *S. aureus*, 98.875% against *P. aeruginosa*; 5% anionic surfactant, didecyl dimethyl ammonium chlorid (120 g/L), octadecyl dimethyl ammonium chloride, hydrogen peroxide, deionized water combination is 100% effective against three bacteria; Ahp: accelerated hydrogen peroxide is 100% effective against *E. coli* and *S. aureus*, 88.462% against *P. aeruginosa*; cationic polymer layer was found to be 100% effective against *E. coli* and *P. aeruginosa*, 99.625% effective against *S. aureus* and deodorizing, general surface cleaning liquid was found to be 100% effective against *E. coli* and *P. aeruginosa*, 98.875% effective against *S. aureus*.

However, when the logarithm differences and antibacterial properties of a total of eight different surface disinfectants were examined as percentages, the logarithm difference of three disinfectants against the three bacteria examined was ≥ 5; the logarithm difference of five disinfectants against two bacteria was ≥ 5; the percentage of those effective against all three bacteria was 37.5%; the percentage of those effective against both bacteria was found to be 62.5% (Table 1).

As a result of our study, it was determined that three surface disinfectants containing 5% anionic surfactant, didecyl dimethyl ammonium chloride (120 g/L), and octadecyl dimethyl ammonium chloride, hydrogen peroxide, and deionized water had logarithm differences ≥5 log after 5 minutes of exposure to the three bacteria used in the analysis, and therefore they were effective. In the studies conducted, the antimicrobial activities of many different disinfectants have been investigated.

Table 1. Logarithm differences and antibacterial properties of different surface disinfectants

Active ingredient	*E. coli*[a]	*E. coli*[b]	*S. aureus*[a]	*S. aureus*[b]	*P. aeruginosa*[a]	*P. aeruginosa*[b]	Antibacterial Property
Active Anionic Oxygen Natural Water	100	≥5 Log	98.875	<5 Log	100	≥5 Log	**
Quaternary Ammonium	100	≥5 Log	100	≥5 Log	98.875	<5 Log	**
%5 Anionic surfactant	100	≥5 Log	100	≥5 Log	100	≥5 Log	***
Didecyl dimethyl ammonium chlorid (120 g/L)	100	≥5 Log	100	≥5 Log	100	≥5 Log	***
Ahp: Accelerated hydrogen peroxide	100	≥5 Log	100	≥5 Log	88.462	<5 Log	**
Cationic polymer layer	100	≥5 Log	99.625	<5 Log	100	≥5 Log	**
Octadecyl Dimethyl Ammonium Chloride, Hydrogen Peroxide, Deionized Water	100	≥5 Log	100	≥5 Log	100	≥5 Log	***
Deodorizing, general surface cleaning liquid	100	≥5 Log	98.875	<5 Log	100	≥5 Log	**

[a]: % Antibacterial Property, [b]: Logarithm difference, **: two bacteria, ***: three bacteria

DISCUSSION AND CONCLUSION

There are few studies on the determiation of the antibacterial activities of the surface disinfectants we used in our study.

In the study of Mataracı and Gerçeker (2011) examined the minimum bactericidal concentrations (MBC values) of SH and benzalkonium chloride of the *P. aeruginosa* ATCC 15442 standard strain against planktonic and biofilm cell cultures by microdilution method under two different experimental conditions: clean and dirty. It has been determined that benzalkonium chloride does not show any significant bactericidal activity against *P. aeruginosa*. It was stated that the type, amount, and contact time of the disinfectant are effective when disinfecting a water system with suspicion of biofilm (Mataracı and Gerçeker, 2011). The three disinfectants examined in our study (5% anyonic surfactant, didecyl dimethyl ammonium chloride (120 g/L) and octadecyl dimethyl ammonium chloride, hydrogen peroxide, and deionized water combination) are stated to be effective. It was found to be effective against the three bacteria examined.

In another study, the activities of three different disinfectants containing sodium dichloroisocyanurate aldehyde, and didecyl dimethyl ammonium chloride were investigated with three different methods. In the study, it was determined that European Suspension Test (EST) was the most convenient and easy-to-apply method among the Modified Kelsey-Skyes, Deutsche Gesellschaft für Hygiene und Mikrobiologie (DGHM), and EST methods. It has been observed that there is a variable agreement between the results of the two methods depending on the disinfectant used and the type of bacteria (Özbek, 1997). As a result of the study, disinfectant containing sodium dichloroisocyanurate was effective against *P. aeruginosa*; disinfectants containing aldehyde and didecyl dimethyl ammonium chloride were found to be ineffective against *P. aeruginosa*. On the other hand, in our study, it was observed that the disinfectant containing didecyl dimethyl ammonium chloride was effective against *P. aeruginosa*.

Kaya and Altanlar, (2021) investigated the antimicrobial activities of disinfectants and antiseptics frequently used in hospitals. 2% glutaraldehyde, 6% hydrogen peroxide solution, and sodium hypochlorite solution (1000 ppm) were used as disinfectants; *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 15542, *S. epidermidis* ATCC 12228, *S. epidermidis* ATCC 35984, methicillin-resistant *S. aureus* (MRSA) ATCC 43300, methicillin-sensitive *S. aureus* (MSSA) ATCC 25923, *P. aeruginosa* ATCC 27853. Antimicrobial activities were investigated by quantitative suspension test against *E. coli* ATCC 25922 reference strains. 2% glutaraldehyde and SH (1000 ppm) showed antimicrobial activity against *P. aeruginosa* ATCC 15542 strain at all contact times (5 minutes, 20 minutes) except 1 minute and against all other strains at all contact times (1 minute, 5 minutes, 20 minutes). 6% hydrogen peroxide solution was not effective against *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 15542, *S. aureus* ATCC 43300 (MRSA), and *S. aureus* ATCC 25923 (MRSA) strains at 1 minute contact time. In our study, it was determined that the surface disinfectant containing octadecyl dimethyl ammonium chloride, hydrogen peroxide, and deionized water was effective against the three bacteria used in the analysis, after 5 minutes of exposure, the logarithm difference was $\geq$5 log and therefore it was effective. Other chemicals used together with hydrogen peroxide may have increased antimicrobial effects.

The selection, effectiveness, reliability, and correct application of disinfectants used to neutralize bacteria that cause infections are very important. Therefore, determining the antibacterial effectiveness of disinfectants and using disinfectants according to these results is necessary to prevent infections. Appropriate disinfectants must be selected to protect consumers and/or healthcare workers from bacterial infections, especially to prevent hospital-acquired infections. The surface disinfectants we found in our study, which are licensed by competent authorities but do not have sufficient antimicrobial activities, will not be able to adequately protect healthcare workers and consumers in terms of hygiene. For this reason, it is necessary to check the antibacterial activities of disinfectants even after the registration stage and to select disinfectants according to their effectiveness to prevent infections.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: S.K, G.E. E.G, Design: S.K, G.E., E.G, Ş.D., Data Collection or Processing: S.K, G.E., E.G, Ş.D., B.G.G., Analysis or Interpretation: S.K., E.M.Ç., A.K., E.G., B.G.G., Literature Search: S.K., N.A., Writing: S.K., N.A.

Financial Support

This research received no grant from any funding agency/sector.

REFERENCES

BS EN 13727: 2012+A2: 2015. Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity in the medical area - Test method and requirements (phase 2, step 1)

Choi HY, Lee YH, Lim CH, Kim YS, Lee IS, Jo JM, Lee HY, Cha HG, Woo HJ, Seo DS. 2020. Assessment of respiratory and systemic toxicity of Benzalkonium chloride following a 14-day inhalation study in rats. Particle and Fibre Toxicology, 17(5):2-19.

Gessi A, Formaglio P, Semeraro B, Summa D, Tamisari E, Tamburini E. 2023. Electrolyzed Hypochlorous Acid (HOCl) Aqueous Solution as Low-Impact and Eco-Friendly Agent for Floor Cleaning and Sanitation. International journal of environmental research and public health. Int. Environ. Res. Public Health, 20(6712): 2-17.

Kameda T, Oka S, Igawa J, Sakamoto M, Terada K. 2022. Can hypochlorous acid be a powerful sanitizer to replace alcohol for disinfection? -Its bactericidal, degradation of the solutions under various storage condition, and steel rust effects. Dental Materials Journal. 41(1):167-183.

Kayan S, Altanlar N. 2021. Investigation of the antimicrobial activities of some disinfectants and antiseptics which are frequently used in hospitals. Journal of Faculty of Pharmacy of Ankara University. 45(2):297-308.

Mataracı E, Gerçeker AA. 2011. Çeşitli dezenfektanların minimum bakterisidal konsantrasyonlarının Pseudomonas Aerugınosa'nın biyofilm kültürlerine karşı araştırılması. ANKEM Derg, 25(4):209-214. Doi: 10.5222/ankem.2011.209

Özbek ÖA. 1997. Yüzey dezenfektanlarının bakterisidal etkinliğini belirlemede kapasite, taşıyıcı ve süspansiyon yöntemlerinin karşılaştırmalı değerlendirilmesi. Dokuz Eylül Üniversitesi Tıp Fakültesi Mikrobiyoloji Ana Bilim Dalı, Tıpta Uzmanlık Tezi, İzmir.

Practice Guidance for Health Care Environmental Cleaning, 3rd eBook, 2022

WHO, 2021. Application for Inclusion in the 2021 WHO Essential Medicines List. Hypochlorous Acid (HOCl) for disinfection, antisepsis, and wound care in Core Categories 15.1, 15.2, and 13. Briotech, Inc. 14120 NE 2ooth St Woodinville, WA 98072 USA. Page: 1-57.