UDC 614.484:579.61

doi: https://doi.org/10.32402/dovkil2026.03.072

BACTERICIDAL EFFICIENCY OF WORKING DISINFECTANT SOLUTIONS

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OBJECTIVE. To investigate the effectiveness of working solutions of disinfectants against resistant microorganisms and microflora of inpatient departments of healthcare facilities.

MATERIALS AND METHODS. The material for the study was working solutions of fifteen commercial disinfectants of different compositions, bacterial cultures (Staphylococcus aureus, Escherichia coli) and microflora of inpatient departments of hospitals. The effectiveness was determined by the Oxford cups method on Mueller-Hinton medium. The study was conducted in duplicate.

RESULTS. The bactericidal effectiveness of working solutions of disinfectants against the tested cultures of microorganisms was different. Thus, the effective working solutions Danaklin 3D and Green Line Basic were found to be effective, and the ineffective ones were Desactin and Lagocid 300. All other tested working solutions of disinfectants showed weak effectiveness.

CONCLUSIONS. The results of our study indicate the importance of conducting such studies, since the effectiveness of the claimed working solutions may decrease over time. This fact is not associated with the development of resistance in microorganisms to disinfectants. It can be explained by the development of protective mechanisms in bacteria (for example, the formation of dry or wet biofilms), which create a certain barrier for the chemically active components of disinfectants. Therefore, we consider it necessary to recommend an increase in the concentration of disinfectants for effective disinfection in healthcare facilities.

KEYWORDS: *disinfectants, effectiveness, Staphylococcus aureus, Escherichia coli, hospital microflora.*

БАКТЕРИЦИДНА ЕФЕКТИВНІСТЬ РОБОЧИХ РОЗЧИНІВ ДЕЗІНФЕКТАНТІВ

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МЕТА. Дослідити ефективність робочих розчинів деззасобів, щодо резистентних мікроорганізмів та мікрофлори стаціонарних відділень закладів охорони здоров'я.

МАТЕРІАЛИ ТА МЕТОДИ. Матеріалом для дослідження були робочі розчини п'ятнадцяти комерційних деззасобів різного складу, бактеріальні культури (Staphylococcus aureus, Escherichia coli) та мікрофлора стаціонарних відділень лікарень. Визначення ефективності проводили за методом Оксфордських чашок на середовищі Мюллера-Хінтона. Дослідження проведене у двох повторностях.

РЕЗУЛЬТАТИ. Бактерицидна ефективність робочих розчинів деззасобів щодо тестованих культур мікроорганізмів була різною. Так, ефективними виявились робочі розчини Данаклін 3Д та Грін лайн базік, а неефективними — Дезактін та Лагоцид 300. Всі інші тестовані робочі розчини деззасобів проявляли слабку ефективність.

ВИСНОВКИ. Результати нашого дослідження вказують на важливість проведення подібних досліджень, так як ефективність заявлених робочих розчинів може з часом знижуватись. Цей факт не пов'язаний із виробленням резистентності у мікроорганізмів до деззасобів. Його можна пояснити виробленням механізмів захисту у бактерій (наприклад, формування біоплівок сухих чи вологих), що створюють певний бар'єр для хімічно-активних компонентів деззасобів. Тому вважаємо за необхідне рекомендувати збільшення концентрації робочих розчинів деззасобів для ефективного проведення дезінфекції у закладах охорони здоров'я.

КЛЮЧОВІ СЛОВА: *деззасоби, ефективність, Staphylococcus aureus, Escherichia coli, лікарняна мікрофлора.*

INTRODUCTION

Antimicrobial resistance is a significant public health threat. The WHO predicts that around 10 million deaths per year will be caused by multidrug-resistant microorganisms [1]. The Global Antimicrobial Resistance and Use Surveillance System collects information and provides conclusions on multidrug-resistant strains of microorganisms based on data from 104 countries around the world. The monitoring covers the eight most common bacterial pathogens: *Acinetobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Neisseria gonorrhoeae*, non-typhoidal *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus* and *Streptococcus pneumoniae*). They are the main cause of infections of the bloodstream, gastrointestinal tract, urinary tract and urogenital system [2].

Mechanical cleaning and chemical disinfection are the most important factors in controlling the spread of pathogens and reducing the number of healthcare-associated infections. Some microorganisms are known to survive for months on dry surfaces [3]. Formulated biocides play a crucial role as disinfectants, controlling microorganisms present on contaminated surfaces, leading to a reduction in the number of pathogens and, consequently, a reduction in the risk of their transmission [4].

New biocidal disinfectants undergo rigorous testing for efficacy according to protocols that should guarantee the product's effectiveness in practical use. Disinfectants approved for use in Ukraine must be included in the «State Register of Disinfectants». It is well known that the effectiveness of disinfectants depends on the test bacteria, as well as the test conditions (planktonic, surface-dried, wet or dry biofilm) and exposure time [4]. It is also necessary to consider the types of antimicrobial tests performed and factors related to the use of the product (contamination, contact time, surface type and target microorganism) [5].

The application technique of some biocidal products may include wiping, which in turn will contribute to the overall effectiveness of the product. *S. aureus* is a common pathogen associated with surfaces and materials in healthcare settings, particularly in inpatient and intensive care units. This organism is responsible for healthcare-associated infections. It is also an important cause of comorbidities in immunocompromised patients [6].

Objective. The objective of our work was to investigate the effectiveness of working solutions of disinfectants used in healthcare facilities against resistant microorganisms and microflora of inpatient departments of healthcare facilities.

MATERIALS AND METHODS

Selection of material for the study. Samples of disinfectants used for routine disinfection in inpatient departments of hospitals were kindly provided by the city's health care facilities. A total of 15 samples of various disinfectants were obtained (*Table 1*).

Filtered tap water was used to prepare working solutions.

Table 1. **Main active ingredients of the tested disinfectants, concentrations, exposure time of working solutions according to the instructions**

№	Means	Composition, active ingredients	Exposure, min.	Action
1	Chlorantine active	Sodium salt of dichlorocyanuric acid 80–98%; adipic acid, sodium bicarbonate, sodium carbonate, sodium sulfate, etc.	30	B, V, F, S, O, L
2	Danoxin special	Sodium percarbonate 15–18%, poly(hexamethylenebiguanide) hydrochloride 0.4–0.5%, etc.	60	B, V, F, S, O, L
3	Terracid premium	Propanediamine, aminopropyldodecyl 2%, phenoxyethanol 5%, alkyldimethylbenzyl ammonium chloride 18%, etc.	60	B, V, F, S, O
4	Desactin	Dichlorodimethylhydantoin 21–23%, dimethylhydantoin 12.4–16.4%, dispersant 9–12%, anionic surfactants 3.2–5%, etc.	60	B, V, F, C
5	Blanidas oxidez	Hydrogen peroxide 12%, alkyldimethylbenzylammonium chloride 3.75%, polyhexamethyleneguanidine hydrochloride 2%, etc.	60	B, V, F, S, O
6	SafeDez	Alkyldimethylbenzylammonium chloride 14–18%, aminopropyldodecyldiamine 14–18%, isopropyl alcohol 5%, excipients, etc.	30	B, V, F, S
7	Danaclin 3D	Alkyldimethylbenzyl ammonium chloride 4.5–5.5%, poly(hexamethylene)guanidine hydrochloride 0.3–0.5%, etc.	30	B, V, F, S
8	Lagocid 300	Dimethylalkylbenzenemethanaminium chloride 15–20%, aminopropyldodecyldiamine 2–6%, etc.	30	B, V, F, S
9	Vitadez EN	Alkyldimethylbenzylammonium chloride 18%, poly(hexamethylene)guanidine hydrochloride 1%, 2-phenoxyethanol 2.5%, enzyme complex 1.3%, etc.	60	B, V, F, S, O, L
10	CLEAN LINE	Aminopropyldodecylpropanediamine 4.5–5.72%, ammonium alkyldimethylbenzyl chloride 2.25–2.85%, isopropyl alcohol 2–2.5%, ethyl alcohol 2–2.5%, etc.	30	B, V, F, S

End of the Table 1

№	Means	Composition, active ingredients	Exposure, min.	Action
11	BacterioDez instru	Alkyldimethylbenzylammonium chloride 16–20%, aminopropyl dodecyl propanediamine 16–20%, isopropyl alcohol 5%, etc.	30	B, V, F, S, O, L
12	Quartacid chlorine active	Trichloroisocyanuric acid 43%, sodium salt of dichloroisocyanuric acid 20%, sodium bicarbonate 10%, sodium carbonate 20%, etc.	60	B, V, F, S
13	Danoxin concentrate	Sodium percarbonate 50–55%, tetraacetylethylenediamine 18–25%, etc.	60	B, V, F, S, O, L
14	Red line activ	Sodium salt of dichloroisocyanuric acid, excipients, active chlorine content not less than 50%	60	B, V, F
15	Green line bazic	Mixture of quaternary ammonium compounds 14.4%, dodecyldipropylenetriamine 0.9%; polyhexamethyleneguanidine 1%, trilon B 0.5%, etc.	30	B, V, F

Note: B — bactericidal; V — virucidal; F — fungicidal; S — sporicidal; O — ovicidal; L — larvicidal.

Samples of hospital microorganisms were obtained from inpatient departments of city hospitals. For this purpose, swabs were taken from surfaces in treatment rooms (10 samples) and wards (10 samples) with a sterile swab (eSwab, Copan, USA) and immediately transported to the laboratory. The tubes with the samples were left in a thermostat for 2 days at a temperature of 37°C. Species identification of microorganisms was not performed.

Samples of resistant microorganisms for the study were obtained in the Microbiological Laboratory of the SE Medical Institute of Sumy State University. Identification of microorganisms was carried out using generally accepted methods based on morphological, tincture, cultural and biochemical properties. The test cultures were 10 samples of *S. aureus* and 10 samples of *E. coli*. Bacterial cultures that were not sensitive to at least 3 antibiotics were considered resistant. A total of 20 samples were examined.

Determination of the sensitivity of microorganisms to basic disinfectant solutions. The study was conducted from 10.2025 to 02.2026 on the basis of the Microbiological Laboratory of the SE Medical Institute of Sumy State University. The antibacterial activity of working disinfectant solutions was assessed by the Oxford cup method.

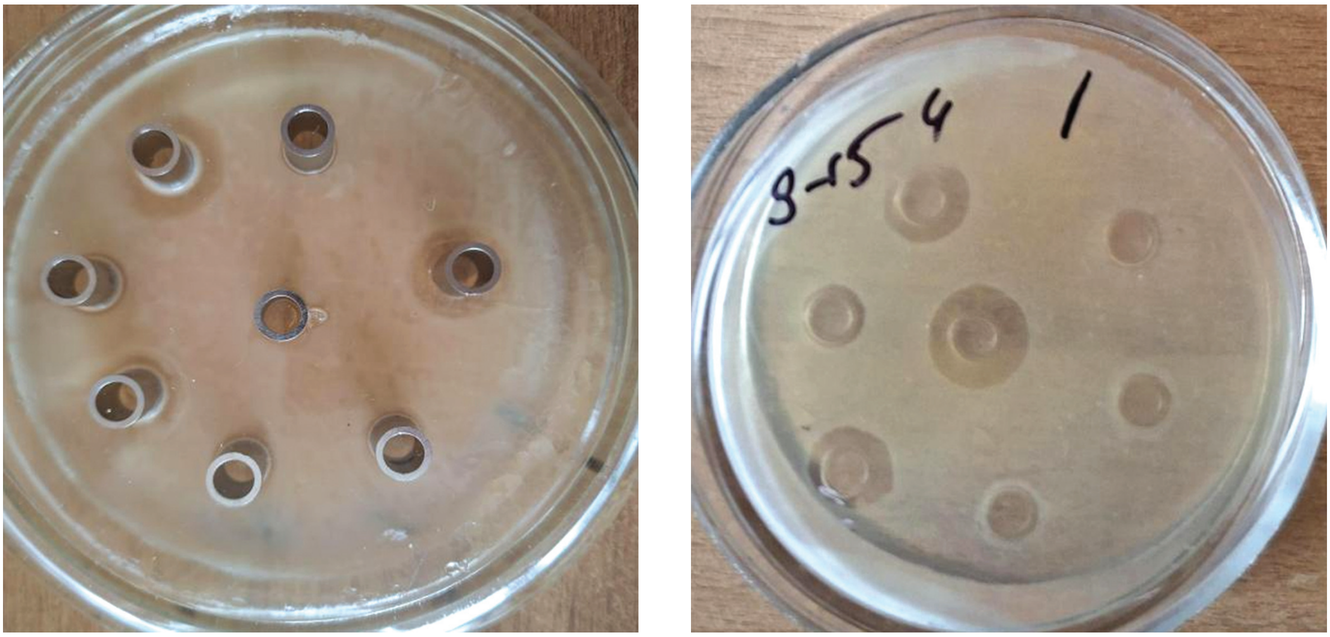

Fig. 1. Testing using the Oxford Cup method

Previously, Oxford cups (stainless steel tubes with an inner diameter of 6 mm, an outer diameter of 8 mm, and a height of 10 mm) (E-commerce Co, China), Mueller-Hinton medium (Farmaktiv LLC, Ukraine), other materials and reagents were sterilized by autoclaving at a temperature of 121°C for 20 min.

After that, the bacterial suspension (about 10^6 CFU/ml) was applied by the lawn method to a Petri dishes on Mueller-Hinton medium. Oxford cups were placed on the surface of the medium (7 pcs.) and freshly prepared working disinfectant solutions (20 µl each) were added. Next, the Petri dishes were placed in a thermostat and incubated at a temperature of 37°C. After 24 hours of incubation, the growth inhibition zone inside and around the Oxford dishes was determined to assess the antibacterial activity of the solutions (see *Fig. 1*). The procedure was performed in duplicate.

RESULTS AND DISCUSSION

According to the effectiveness of the action, the working solutions of disinfectants were divided into 3 groups — effective (pronounced zone of growth inhibition around the cups), weakly effective (growth inhibition within the cup) and ineffective (no growth inhibition). As a result of the conducted studies, it was found that the tested cultures showed similar sensitivity to the working solutions of disinfectants (*Table 2*).

Thus, the flora of hospital premises was sensitive to Blanidas oxidez, and *S. aureus* and *E. coli* showed average sensitivity. At the same time, the working solution Danoxin concentrate was ineffective against *S. aureus* and *E. coli* and weakly effective against hospital microflora.

According to the results, the microflora of treatment rooms was less sensitive to the action of almost all working disinfectant solutions than the microflora of hospital wards.

Chemical disinfectants used in healthcare facilities for routine disinfection of premises (surfaces) can be conditionally divided into chlorine-containing and non-chlorine-containing. It should be noted that the main active ingredient in 10 of the 15 solutions studied was chlorine. The content of active chlorine is of great importance in the preparation and subsequent use. Hypochlorites, the most common chlorine disinfectants, are available in liquid (e.g., sodium hypochlorite) or solid form (e.g., calcium hypochlorite). They have a wide spectrum of antimicrobial activity, do not leave toxic residues, are independent of water hardness, are inexpensive and fast-acting [7] and have low toxicity, but exhibit corrosive effects and are relatively stable.

Table 2. **Sensitivity of microorganisms to working solutions of disinfectants**

№	Means	%	***S. aureus*, *n* = 10**	***E. coli*, *n* = 10**	**Treatment room microflora, *n* = 10**	**Ward microflora, *n* = 10**
1	Chlorantine active	0,03	+/–	+/–	+/–	+/–
2	Danoxin special	0,05	–	–	–	–
3	Terracid premium	0,05	+	+/–	+/–	+/–
4	Desactin	0,10	–	–	–	–
5	Blanidas oxidez	0,10	+/–	+/–	+	+
6	SafeDez	0,05	+/–	+/–	+/–	+
7	Danaclin 3D	0,25	+	+	+	+
8	Lagocid 300	0,02	–	–	–	–
9	Vitadez EN	0,05	+	+/–	+	+
10	CLEAN LINE	0,10	+/–	+/–	+/–	+
11	BacterioDez instru	0,03	+	+/–	+/–	+
12	Quartacid chlorine active	0,02	+/–	+/–	+/–	+/–
13	Danoxin concentrate	0,05	–	–	+/–	+/–
14	Red line activ	0,05	+/–	+/–	+/–	+/–
15	Green line bazic	0,10	+	+/–	+	+

Note: + — effective; +/– — weakly effective; – — ineffective.

In our study, not all chlorine-containing agents in working solutions showed the same activity, which we primarily associate with the content of active chlorine. Thus, the working solutions Chlorantin active, Quartacid chlorine active and Red Line active showed weak effectiveness against all tested microorganisms. Terracid premium showed high effectiveness against *S. aureus*, and CefDez — against the microflora of hospital wards. BacterioDez instru had high effectiveness against *S. aureus* and the microflora of the wards.

Among the tested chlorine-containing disinfectants, 2 were found, the working solutions of which were ineffective against all tested cultures — Desactin and Lagocid 300. This effect can be explained by the low concentration of the working solution, the low content of active chlorine, or the adaptive ability of microorganisms through the formation of biofilms. It is known that *S. aureus* complicates the disinfection process due to the possibility of forming dry surface biofilms [8], and bacteria can also develop increased tolerance or resistance to chlorine-containing disinfectants, but are unable to develop resistance to chlorine [9].

Working solutions of disinfectants based on sodium percarbonate — Danoxin special and Danoxin concentrate — were equally ineffective against resistant *S. aureus* and *E. coli*, but Danoxin concentrate showed average effectiveness against the microflora of hospital facilities. After dissolving in water, sodium percarbonate releases hydrogen peroxide, acting as a powerful oxidant. This makes it quite effective for removing various pathogenic microorganisms — bacteria, viruses, fungi [10]. The low effectiveness of the above working solutions in our study is most likely due to their insufficient concentration.

The working solution of Green Line basic was effective against all tested samples, although *E. coli* was still less sensitive. The basis of this disinfectant is a mixture of quaternary ammonium compounds that interact with several target sites of microorganisms. The mechanisms of their antimicrobial action are disruption of the cell membrane, inhibition of the aerobic respiratory system of microbes, precipitation of cytoplasmic material and deactivation of the protective lipid shell [11].

It should be noted that all the disinfectants studied, except for Chlorantin active (up to 80% active Cl) and Redline active (up to 50% active Cl), were mixtures of different chemically active substances. These substances are able to exhibit a synergistic effect, enhancing the action of disinfectants [12].

CONCLUSIONS

Our study revealed certain shortcomings in the effectiveness of working solutions of disinfectants. The results indicate the importance of conducting such studies, since the effectiveness of the declared solutions may decrease over time. This fact is not associated with the development of resistance in microorganisms to disinfectants. It can be explained by the development of defense mechanisms in bacteria (for example, the formation of dry or wet biofilms), which create a certain barrier for the chemically active components of disinfectants. Therefore, we consider it necessary to recommend increasing the concentration of disinfectant solutions for effective disinfection in healthcare facilities.

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FUNDING

No financial support was received from any individuals or organizations for the conduct or publication of this work. This work was carried out as part of the master's research «Evaluation of the effectiveness of basic disinfectant solutions against multiresistant microorganisms and flora of inpatient departments of hospitals in the city of Sumy».

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

USE OF GENERATIVE ARTIFICIAL INTELLIGENCE

Generative artificial intelligence was not used in the writing of this article.

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Отримано: 13.08.2026
Прийнято до друку: 17.09.2026
Опубліковано: 30.09.2026

ФІНАНСУВАННЯ

Фінансової підтримки від жодних осіб чи організацій для проведення або публікації цієї роботи не було отримано. Ця робота виконана в рамках магістерського дослідження «Оцінка ефективності базових дезінфекційних розчинів проти мультирезистентних мікроорганізмів та флори стаціонарних відділень лікарень міста Суми».

КОНФЛІКТ ІНТЕРЕСІВ

Автори заявляють про відсутність конфлікту інтересів.

ВИКОРИСТАННЯ ГЕНЕРАТИВНОГО ШТУЧНОГО ІНТЕЛЕКТУ

Генеративний штучний інтелект не використовувався у написанні цієї статті.

ВІДОМОСТІ ПРО АВТОРІВ ТА ЇХ ВНЕСОК

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Received: 13.08.2026
Accepted: 17.09.2026
Published: 30.09.2026