

# Effectiveness of hydrogen peroxide wipes for surface disinfection in healthcare facilities

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## Abstract

**Introduction.** The correct method of surface disinfection in hospitals is an essential tool in the fight against the spread of healthcare-associated infections caused by multi-resistant microorganisms. Currently, there are many disinfectants on the market that can be used against different microorganisms. However, the effectiveness of different active molecules is controversial in the literature.

**Study design.** The aim of this study was to evaluate the effectiveness of wipes based on hydrogen peroxide (1.0 %) and highly specific plant-based surfactants, contained in H<sub>2</sub>O<sub>2</sub><sup>TM</sup> (Hi-speed H<sub>2</sub>O<sub>2</sub><sup>TM</sup>) products, against some hospital-associated microorganisms.

**Methods.** The effectiveness of the wipes was tested against nosocomial and control strains of methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* carbapenemase, *Aspergillus fumigatus* and *Candida parapsilosis*. Specifically, *in vitro* activity was assessed using three different techniques: stainless steel surface testing, surface diffusion testing and well diffusion test.

**Results.** The three different methods tested confirm the wipes' good effectiveness against the most common multi-resistant bacteria and against fungi.

**Conclusions.** These data show that the tested wipes could be a valid adjunct to the disinfection process and could assist in the prevention of healthcare-associated infections.

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## Introduction

Healthcare-associated infections (HAIs) are the most common adverse events worldwide, causing significant morbidity, mortality and financial burden to patients and the healthcare systems (1). The European Center for Disease Prevention and Control (ECDC) estimates that more than 3.5 million cases of HAIs occur in the European Union and European Economic Area (EU/EEA) each year, resulting in more than 90,000 deaths and approximately 2.5 million Disability Adjusted Life Years (DALYs). In the EU/EEA, this burden is estimated to be greater than the cumulative burden of other infections, including influenza and tuberculosis. Furthermore, 71% of HAIs are caused by bacteria that are resistant to antimicrobials, including bacteria that are resistant to final-line antimicrobials, such as carbapenem-resistant *Enterobacteriaceae* (2).

In addition to respiratory, fecal-oral and sexual transmission, the transfer of pathogens via surfaces also plays an important role in human infections (3,4). In hospitals, the probability of microbial environmental spread can be influenced by the tenacity of the circulation of microorganisms and the presence of immunocompromised subjects (5,6). The Worldwide Outbreak Database (7) is the largest collection of nosocomial epidemics. According to this database, the bacteria that play a main role in epidemic events are *Staphylococcus aureus* (11.9%), *Klebsiella pneumoniae* (7.9%) and *Pseudomonas aeruginosa* (7.1%), followed by methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile*, vancomycin-resistant *Enterococcus* (VRE) and *Acinetobacter* spp. (8). These microorganisms can persist in the environment for hours to days (and in some cases for months), especially if the circulating bacteria are *Klebsiella pneumoniae* (from 2 hours to 30 months) and *Pseudomonas aeruginosa* (from 6 hours to 16 months) (9). Their movement is facilitated by the inadequate use of personal protective equipment by healthcare workers. In fact, healthcare workers have frequent contact with the equipment present in patients' rooms (accessories, bed, bedside table, door or window handles), so they can easily contaminate their hands or gloves. In addition, they can transmit microorganisms using mobile phones, as well as through the use of computers during healthcare activities or surgical procedures (10). According to Paleckyte et al. (11), the management of control measures by healthcare workers is also associated with multi-resistant bacteria. The lack of education

and training on infection control policies and other essential working practices remain a major barrier to the effective implementation of control measures.

Among the different prevention methods necessary to reduce the risk of infections in healthcare settings, disinfection plays an essential role. The intervention must be carried out by choosing the disinfectants that best meet the needs of use. These products, depending on the mechanism of action, can block the reproduction of the microorganism (bacteriostatic action) or prevent it completely (bactericidal, virucidal, fungicidal or sporicidal action). Their effectiveness and speed of action are linked to various factors including the type of disinfectant adopted, the conditions of use, the microbial species on which to act, the presence of organic substance. Also, in daily practice, method of use, concentration, contact time, presence of inactivating substrates can largely influence the effectiveness of a disinfectant, influencing the expected level of disinfection. For example, if a high-level disinfectant (i.e. active across the entire microbial spectrum, except for spores present in high concentrations) is used at concentrations lower than the effective ones or for an insufficient contact time or in the presence of substances that interfere with the action of the active components, certainly it does not provide the expected results.

In recent years, there has been a growing consensus on the need for improvement in the cleaning and disinfection of surfaces in healthcare facilities (12).

During the COVID-19 pandemic, interest in contrasting microbial contamination of surfaces has increased significantly both in the community setting (13-17) and in the healthcare setting (18-20). Some authors have reported that SARS-CoV-2 is transmitted by touching surfaces on which a sick person has recently coughed or sneezed (21-23). Rooms occupied by patients with multidrug-resistant organisms, if not adequately disinfected, can represent a relevant risk for transmission to other patients using the same room (24). Thorough cleaning and/or disinfection of surfaces, especially at the time of patient discharge, are essential elements for an effective prevention program. It is mandatory not only to use disinfectants appropriately, but they must be effective (biocides) on a broad spectrum of microorganisms if the risk of patients developing infections from healthcare-associated pathogens is to be reduced (25).

Among different disinfection products generally used in the healthcare setting, the action of hydrogen peroxide is particularly interesting for its bactericidal, virucidal, sporicidal, and fungicidal properties (26,

27). It is an oxidizing agent that works by producing free hydroxyl radicals that can attack membrane lipids, DNA, and other essential cellular components. Oxidizing agents are used for hard surface disinfection and high-level disinfection of medical devices (28). Among the main advantages, hydrogen peroxide has broad-spectrum activity as a biocide, which includes effectiveness against bacterial endospores. Furthermore, its decomposition does not produce toxic by-products (29).

Although hydrogen peroxide has been used for many years as a disinfectant, Bharti et al. 2022 (30) underline that this molecule releases oxygen over time as the product formed after the decomposition is the mixture of hydrogen and water.

In 2015, a new formulation of 1.0% hydrogen peroxide impregnated wipes (Incidin™ Oxy Wipe, Ecolab Deutschland GmbH, Monheim am Rhein, Germany) was first developed and launched in the United States. It was called “enhanced” or “accelerated” with Hi-speed H<sub>2</sub>O<sub>2</sub> because it allows hydrogen peroxide to penetrate microorganisms faster and more efficiently and can be used as a ready-to-use cleaner and disinfectant against bacteria and viruses. Recently, these wipes have been introduced in Italy (Incidin™ Oxy Wipe, produced by Ecolab srl, Vimercate - MB, Italy).

The aim of this study is to evaluate the effectiveness of Incidin™ Oxy Wipe, whether in the form of wipes or liquid disinfectant, against some microorganisms of nosocomial origin using different laboratory techniques in order to verify whether different methods confirm the same results.

## Methods

The effectiveness of Incidin™ Oxy Wipe wipes (dimensions 20 x 20 cm) made of viscose (40%) and polyethylene terephthalate (60%) was tested against bacteria and fungi (specifically, five strains of nosocomial origin and five reference strains) divided into three different groups:

Group A (nosocomial strains subjected to disinfectant treatment): methicillin-resistant *Staphylococcus aureus* (MRSA), Carbapenem-resistant *Pseudomonas aeruginosa* (CR-PA), *Klebsiella pneumoniae* carbapenemase (KPC), *Aspergillus fumigatus* and *Candida parapsilosis*.

Group B (reference strains subjected to disinfectant treatment): *P. aeruginosa* (NCTC 10662) and *S. aureus* (NCTC 6571) provided by the National Collection of

Type Cultures; *K. pneumoniae* (ATCC 43816), *A. fumigatus* (ATCC 46645), and *C. parapsilosis* (ATCC 22019) provided by the American Type Culture Collection.

Group C (control strains: reference and nosocomial strains not treated with disinfectant): group A (*Staphylococcus aureus*, MRSA; *Pseudomonas aeruginosa*, CR-PA; *Klebsiella pneumoniae*, KPC; *Aspergillus fumigatus* and *Candida parapsilosis*) and group B (*P. aeruginosa* NCTC 10662; *S. aureus* NCTC 6571; *K. pneumoniae* ATCC 43816; *A. fumigatus* ATCC 46645 and *C. parapsilosis* ATCC 22019).

The strains of nosocomial origin were selected from stock cultures preserved in glycerol at -80°C at the Hygiene Laboratory of the University of Bari Aldo Moro. Neither ethical approval nor patient consent was deemed necessary, as we did not use patient data or additional samples beyond those obtained during routine laboratory work.

To ensure the viability and purity of the bacterial strains, each strain was plated on Petri dishes containing Brain-Heart Infusion Agar (BHIA; Biokar Diagnostics, Beauvais, France). After incubation for 24 hours at 36°C ± 1°C, individual colonies were subcultured onto Triple-Sugar-Iron agar (TSI, Biolife Srl, Milan, Italy) and incubated for 24 hours at 36°C ± 1°C. The same procedure was performed with the fungal strains, using Petri dishes containing Sabouraud gentamicin-chloramphenicol agar and incubating at 25°C for 24-48 hours (*C. parapsilosis*) and for five days (*A. fumigatus*).

The study was conducted using three different methods, and the tests were repeated three times for each method and each strain.

### 1. Method I (stainless steel surface test)

Stainless steel sheets (42 cm<sup>2</sup> each) were plated via sterile cotton swabs with 200 µl of each bacterial or fungal suspension (in saline solution) at a concentration of 0.5 McFarland (approximately 1.5 x 10<sup>8</sup> cfu/mL). After spreading the suspensions, the plates were dried at 30 °C for 1 hour to promote adhesion of the bacteria/fungi to the surface. Immediately after incubation, IOW wipes were streaked for 5 seconds onto the steel surface contaminated with Group A and B microorganisms, while Group C microorganisms were not treated.

For each plate of A and B groups, a sterile swab was smeared on the contaminated surface, then suspended in 10 ml of neutralization solution (Easy Surface Checking-Neutralization Rinse Solution; Liofilchem Srl, Roseto degli Abruzzi, Italy) to block the action

of the disinfectant.

According to UNI EN ISO 4833-1:2013 (31), for the determination of the Total Bacterial Count (TBC), 1 mL of neutralization solution of each suspension and the corresponding dilutions were mixed and plated on Plate Count Agar (Microbiol Snc, Cagliari, Italy). They were incubated at  $30 \pm 1$  °C and monitored daily for  $72 \pm 3$  hours.

According to NF V08-059:2002 (32), for the determination of Total Fungal Count (TFC), 1 mL of neutralization solution and each dilution were mixed with Sabouraud gentamicin-chloramphenicol agar (Liofilchem, Roseto degli Abruzzi, Italy). The samples were incubated at  $25 \pm 2$  °C and monitored for 5 days.

Although the group C microorganisms did not meet the disinfectant, swabs with neutralizer were also used on these plates to standardize the methods used.

After incubation, the presence of colonies was expressed as colony forming units per cm<sup>2</sup> (cfu/cm<sup>2</sup>).

The arithmetic mean of each test per microorganism was used to calculate the inhibitory effect of the test product.

## 2. Method II (surface diffusion test)

Surface diffusion tests were performed in 90 mm diameter Petri dishes containing Wurtz lactose agar for bacteria, and Sabouraud gentamicin-chloramphenicol agar (Liofilchem, Roseto degli Abruzzi, Italy) for Fungi. Each plate was thoroughly inoculated with sterile swabs that had been soaked in the respective bacterial and fungal suspensions at a concentration of 0.5 McFarland (approximately  $1.5 \times 10^8$  cfu/mL).

Meanwhile, under sterile conditions, 20 mm diameter wipe discs were prepared and then placed on the surface of each inoculated plate. Before starting the experiment, we cut discs of different diameters (5 mm, 10 mm, 20 mm) from the wipe under sterile conditions. The results were comparable, but we opted for the 20 mm disc because the inhibition zone was more delineated and easily measurable. Furthermore, given the filamentary structure of the wipes, making 20 mm discs was easier.

Plates inoculated with bacteria were incubated for  $72 \pm 3$  h at  $30 \pm 1$  °C, those inoculated with fungi for 5 days at  $25 \pm 2$  °C. The effectiveness of the test was evaluated by measuring the diameter of the microbial inhibition zone around the discs. Microorganisms were considered susceptible when the zone of inhibition was  $> 28$  mm in diameter. This value is given by the

diameter of the disc (20 mm) plus an inhibition of four mm to the left and to the right of the disc.

## 3. Method III (well diffusion test)

The discs were removed to evaluate the presence or absence of underlying growth (33).

In agreement with other authors (34,35) we wanted to carry out the diffusion test in the well to evaluate the effectiveness of the product to be studied, making some modifications. This test was performed in 90 mm diameter Petri dishes containing Wurtz lactose agar for bacteria, and Sabouraud gentamicin-chloramphenicol agar (Liofilchem, Roseto degli Abruzzi, Italy) for Fungi. A direct suspension of colonies of each test isolate was prepared in sterile 0.9% saline solution. Turbidity was adjusted to 0.5 McFarland (approximately  $1.5 \times 10^8$  cfu/mL). Agar plates were thoroughly inoculated with each test suspension by swabbing.

For each plate, three wells were made, one larger (diameter 10 mm) and two smaller (diameter 5 mm), filled respectively with 100 µl and 50 µl of disinfectant liquid obtained by squeezing and twisting the wipes.

The reason that led us to apply two smaller holes is that their sum corresponds to a large hole and therefore we can understand if the inhibiting effect is achieved with both two half doses and a full dose of disinfectant. The effectiveness of the test was evaluated by measuring the diameter of the microbial inhibition zone around the well. Microorganisms were considered sensitive when the zone of inhibition had a diameter  $> 7$  mm for small holes and  $> 14$  mm for large holes. For the small one, a 5 mm diameter hole was considered containing 50 mcL plus 1 mm on the right and 1 one the left with a total diameter of 7 mm, while for the large one, as there was a double quantity of disinfectant (100 mcL), the limit was set at 14 mm. The plates for bacteriological investigations were incubated at  $30 \pm 1$  °C for  $72 \pm 3$  h, while for the mycological ones at  $25 \pm 2$  °C for 5 days.

In order to obtain the certainty of the results from the two repetitions, the values from the two small wells were expressed as an average value.

## Results

The results are given below for each of the individual methods and refer to the mean value obtained from the tests carried out in triplicate.



Table 1 - Results obtained from the stainless steel surface test (*Method I*), expressed as the average value of three time for each strain tests.

Tested strains	Surfaces treated with wipes H <sub>2</sub> O <sub>2</sub>		Surfaces no treated with wipes H <sub>2</sub> O <sub>2</sub>
	Group A	Group B	Group C
	Nosocomial strains	Reference strains	Control
	(cfu /cm <sup>2</sup> )	(cfu /cm <sup>2</sup> )	(cfu /cm <sup>2</sup> )
<i>Staphylococcus aureus</i> (MRSA)	0	0	260
<i>S. aureus</i> (NCTC 6571)	0	0	270
<i>Pseudomonas aeruginosa</i> (CR-PA)	0	0	250
<i>P. aeruginosa</i> (NCTC 10662)	0	0	280
<i>Klebsiella pneumoniae</i> (KPC)	0	0	280
<i>K. pneumoniae</i> (ATCC 43816)	0	0	300
<i>Aspergillus fumigatus</i>	0	0	330
<i>A. fumigatus</i> (ATCC 46645)	0	0	350
<i>Candida parapsilosis</i>	0	0	290
<i>C. parapsilosis</i> (ATCC 22019)	0	0	300

### 1. Method I (stainless steel surface test)

Incidin™ Oxy Wipe wipes soaked in H<sub>2</sub>O<sub>2</sub> resulted effective on all strains tested in triplicate (100%): the strains of Group A (nosocomial strains) and B (reference strains) treated with H<sub>2</sub>O<sub>2</sub> wipes produced negative results (0 cfu /cm<sup>2</sup> each). On the contrary, the Group C strains (control strains) tested as controls developed colonies with a bacterial load between 250 and 350 cfu/cm<sup>2</sup> (Table 1).

### 2. Method II (surface diffusion test)

All the strains examined presented an inhibition zone > 28 mm in diameter, therefore they were all

considered sensitive to the action of the disinfectant. However, a difference in inhibition values between bacteria and fungi was detected. In particular, MRSA strains were the most sensitive (40 mm), followed by KPC (31 mm), *P. aeruginosa* (30 mm), *A. fumigatus* and *C. parapsilosis* (30 and 29 mm, respectively). When the discs were removed, no bacterial or fungal growth was detected. An example of the surface diffusion test is shown in Figure 1.

Group C strains (control strains) tested as controls, not having come into contact with the disinfectant, didn't register any inhibition (Table 2).

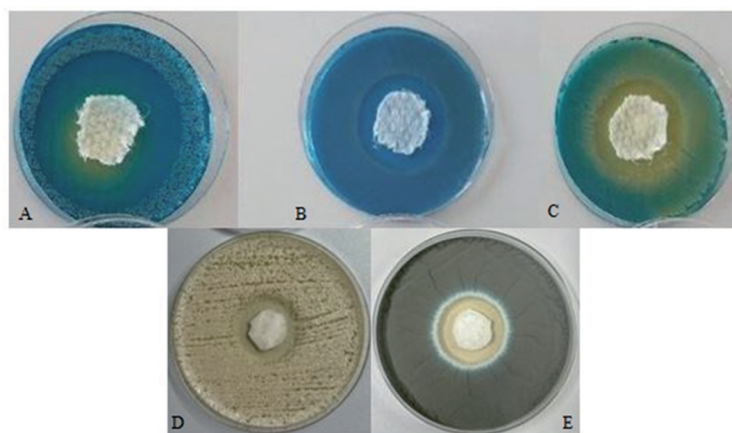


Figure 1 - Inhibition halos of bacterial and fungal growth on the strains tested: *Staphylococcus aureus* (MRSA) (A), *Pseudomonas aeruginosa* (CR-PA) (B), *Klebsiella pneumoniae* (KPC) (C), *Candida parapsilosis* (D) and *Aspergillus fumigatus* (E).

Table 2 - Results obtained from the surface diffusion test (*Method II*), expressed as the average value of three time for each strain tests.

Tested strains	Group A e B treated with wipe discs	Group C, as control
	Inhibition growth (mm)	Inhibition growth (mm)
<i>Staphylococcus aureus</i> (MRSA)	40 mm	0 mm
<i>Pseudomonas aeruginosa</i> (CR-PA)	30 mm	0 mm
<i>Klebsiella pneumoniae</i> (KPC)	31 mm	0 mm
<i>Aspergillus fumigatus</i>	30 mm	0 mm
<i>Candida parapsilosis</i>	29 mm	0 mm

### 3. Method III (well diffusion test)

Satisfactory results were obtained with both 5 mm and 10 mm diameter holes. The inhibitory effect obtained from the two smaller holes is roughly equivalent to the inhibitory effect obtained from one large hole. In fact, after repeating this method three times, the average values deriving from the inhibition zone measurements in triplicate were calculated: *S. aureus* MRSA was the most sensitive (mean value of big and of two small hole 30 mm), followed by *P. aeruginosa* CR-PA and *Klebsiella* KPC (mean value of big and two small hole 18 and 15 mm, respectively). As regards the fungal strains, *Candida parapsilosis* was more sensitive (big hole 20 mm, mean value of the two small hole 10 mm) than *Aspergillus fumigatus* (big hole 12 mm, mean value of the two small hole 8 mm) (Figure 2).

### Discussion

The effective use of disinfectants is part of a multibarrier strategy to prevent HAIs. The surfaces are generally considered non-critical items because they meet intact skin. Therefore, contact with surfaces, although in a healthcare environment, is wrongly considered to pose minimal risk of causing infection in patients or nosocomial staff. Even today, the routine use of germicidal substances to disinfect hospital surfaces and other non-critical objects are object of debate across the world (36, 37).

Indeed, environmental surfaces can potentially contribute to cross-transmission of HAIs. Some authors have pointed out that it is easy to transfer microorganisms from the hands or gloves of healthcare workers to patients and from patient to patient, because

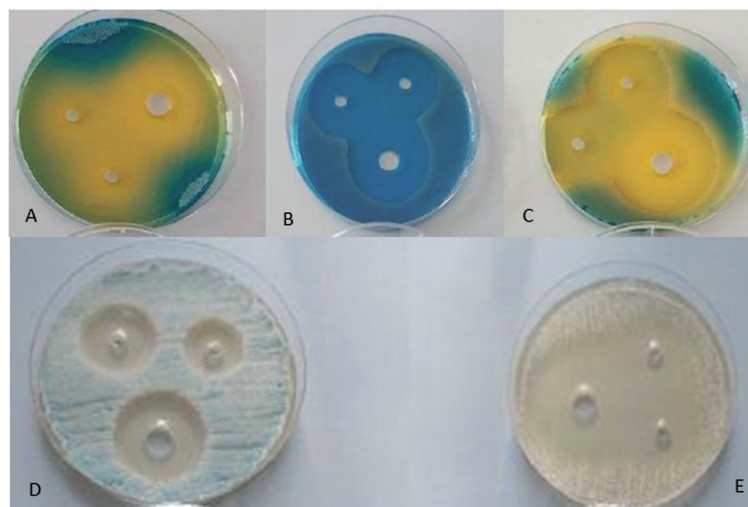


Figure 2 - Inhibition halos of bacterial and fungal growth on the strains tested: *Staphylococcus aureus* (MRSA) (A), *Pseudomonas aeruginosa* (CR-PA) (B), *Klebsiella pneumoniae* (KPC) (C), *Aspergillus fumigatus* (D) and *Candida parapsilosis* (E).

Table 3 - Results obtained from the well diffusion test (*Method III*), expressed as the average value of three time for each strain tests on big and small hole.

Tested strains	Group A e B treated with wipes H <sub>2</sub> O <sub>2</sub>		Group C, as control
	Inhibition growth (mm)		Inhibition growth (mm)
	Big hole	Small hole	
<i>Staphylococcus aureus</i> (MRSA)	30	30	0
<i>Pseudomonas aeruginosa</i> (CR-PA)	18	15	0
<i>Klebsiella pneumoniae</i> (KPC)	18	15	0
<i>Aspergillus fumigatus</i>	12	8	0
<i>Candida parapsilosis</i>	20	10	0

the healthcare worker's contact with the contaminated environment is as likely as the direct contact with a patient (25, 38). Likewise, all the equipment usually used in hospitals for patient care (e.g., X-ray machines, instrument trolleys, sphygmomanometers, stethoscopes, electronic thermometers), including walls, tabletops, bedside tables, bed bars and header, mobile phones, personal computers, etc., can be contaminated and, consequently, represent a potential source of infection (39).

An article researched epidemiological and microbiological data regarding the use of disinfectants on non-critical surfaces (40). Other meta-analysis studies (41, 42) have shown that patients admitted to hospital are more likely to contract nosocomial infections if the room had previously been occupied by HAI-positive patients (43, 44).

Surfaces represent a real and important source of transmission of pathogenic microorganisms in hospitals (14, 45), therefore careful disinfection leads to a decrease in surface contamination and to the reduction of HAIs (25, 46).

Various factors such as the characteristics of the built environment, the circulation of staff, patients, and visitors can increase the type and quantity of microorganisms present in the environment and lead to cross contamination (39). Also, climatic conditions (in particular, the degree of humidity) can influence the survival of environmental microorganisms (47, 48).

Considering these issues, surface disinfection becomes a fundamental infection prevention practice. Scientific evidence (25, 49) has shown that appropriate surface disinfection is a key practice in reducing the incidence of HAIs, as conventional disinfection procedures performed with inappropriate products do not always eliminate pathogens from the environment.

Numerous products are listed in the guidelines for disinfection and sterilization in healthcare.

Among these, hydrogen peroxide is one of the most effective (50). These data are consistent with a study that evaluated the in vitro antibacterial activity of five disinfectants used in hospital practice (phenolic compounds, quaternary ammonium compounds, sodium hypochlorite, alcoholic compounds, hydrogen peroxide). Hydrogen peroxide was the most active against both clinical isolates (*K. pneumoniae* sensitive and resistant to carbapenems, MRSA, *P. aeruginosa*, *Enterococcus faecalis*) and environmental isolates (*P. aeruginosa*) (33). Other studies have evaluated no-touch automated room disinfection (NTD) systems. The most used in healthcare facilities are hydrogen peroxide aerosol systems, H<sub>2</sub>O<sub>2</sub> vapor systems, and ultraviolet C radiation systems (51, 52). Some authors (53) have evaluated the bactericidal activity of products based on 0.5% hydrogen peroxide, both alone and in combination with other molecules with disinfectant activity. The study was carried out on stainless steel surfaces against Gram-positive and Gram-negative bacteria. The best results were obtained when the molecule was tested in combination with other antimicrobial products against *Enterococcus hirae* and *P. aeruginosa* compared to *S. aureus*.

In recent years, the use of ready-to-use disinfectants in the form of pre-moistened wipes has become widespread (54). These wipes are made of different materials to allow the disinfectant to act differently on different surfaces (23, 24). Kelley et al. (55) tested five wipes with different contact times (30 seconds, one minute, two minutes, three minutes, and 10 minutes), one impregnated with 0.5% hydrogen peroxide and four based on quaternary ammonium compounds at different concentrations. Only the hydrogen peroxide impregnated wipes were more effective against *S. aureus* and *P. aeruginosa*. It was hypothesized that hydrogen peroxide performed better due to the shorter contact time (1 minute) compared to quaternary ammonium impregnated wipes (55).

The disinfection process using a wipe impregnated with a disinfectant can be divided into two parts (mechanical action and disinfectant action) which make up the overall decontamination activity. The wipes include a cleaning process by mechanical action, which is performed by the healthcare worker and is capable of removing organic dirt and at the same time acting as a disinfectant. It is important to consider that during the rubbing process with the wipe, some microorganisms may simply be transferred from one part of the surface to be treated to another, rather than being removed. This mechanical action depends on the retention capacity of the wipe and the bactericidal activity of the disinfectant adsorbed on the wipe, including the intrinsic properties of the wipe such as surface energy, fabric structure and fiber type, as well as the pressure applied, the number of steps and the type of microbial adhesion mechanism (54, 56). In addition, the bactericidal activity is mainly due to the disinfectant solution that the type of wipe can release onto the surface. Depending on the interaction between the wipe and the disinfectant, the amount and concentration of the active ingredient, the absorbency of the wipe and the amount of solution released onto the surface are important predictors of effectiveness.

To the best of our knowledge, our study is the first scientific contribution evaluating the effectiveness of wipes impregnated with 1.0 % hydrogen peroxide (Incidin™ Oxy Wipe) in the “enhanced” and “accelerated” formulations (Hi-Speed H<sub>2</sub>O<sub>2</sub>™) and containing highly specific plant-based surfactants. This product allows the hydrogen peroxide to penetrate microorganisms faster and more efficiently. The study was conducted on both nosocomial bacteria known to be multidrug-resistant and fungi, using three different methods. All the results confirmed the effectiveness of this molecule on the strains tested, with no differences between the nosocomial and reference strains (ATCC and NCTC). If we consider the product data sheet, Incidin™ Oxy Wipe leaves no toxic residue after use as it decomposes into oxygen and water, without any risk to the user or the environment. Furthermore, the product is considered an effective cleaning agent, presents no health risks for operators, requires short contact times with surfaces and has excellent compatibility with materials. These latter claims were not the subject of our study and, to our knowledge, are not supported by other experiments. It is our intention to expand this investigation, increasing the number of strains to be tested, including other microorganisms responsible for HAIs such as *Acinetobacter baumannii*, *E. coli*, *Serratia marcescens*, *Clostridium difficile* and

vancomycin-resistant *Enterococcus* and verifying their effectiveness on other types of surfaces normally present in healthcare facilities (e.g. glass, wood and plastic). However, in our opinion, the introduction of Incidin™ Oxy Wipe into common disinfection procedures could contribute to reducing the number of hospital infections, with a reduced consumption of antibiotics planned in the therapeutic protocols and a consequent reduction in healthcare costs. Furthermore, the use of pre-impregnated wipes allows us to reduce the quantity of water and disinfectant solutions that are thrown into the sewage every day (57).

In addition to laboratory research, we would like to verify the effectiveness of the wipes directly on ward surfaces and investigate environmentally sustainable disinfection techniques that are effective against multi-resistant microorganisms. Considering that these studies are scarce in the literature (58), it will be necessary in the near future to enhance research on the effectiveness of disinfectants in hospitals to reduce the incidence of cross-contamination and avoid chemical damage to patients and healthcare workers.

## Conclusions

The role of the hospital environment in the transmission of HAIs is still debated across the world. However, scientific evidence supports the hypothesis that, in addition to hand disinfection, surface disinfection is one of the most important prevention tools to limit the transmission of pathogens in healthcare facilities. Surfaces in the immediate vicinity of the patient and surfaces with high hand contact or frequent skin contact should be disinfected regularly. It is important to observe proper protocols such as the use of the appropriate disinfectant, the correct dosage, complete wetting, and exposure times, without neglecting the practicality of the method to be used depending on the circumstances; otherwise, disinfection could be less effective.

Our study demonstrates that the Incidin™ Oxy Wipe 1.0 % hydrogen peroxide-based wipes have an evident and significant antimicrobial action against all the microorganisms examined (Gram positive and Gram-negative bacteria, Fungi). The different methods used confirmed the same results.

These data underline that the tested wipes can exert an effective disinfectant action in the healthcare environment and represent a valid aid in the prevention of HAIs, especially against multi-resistant microorganisms.



This environmental remediation action could be used as a prevention tool in indoor environments, especially where disinfection processes can be particularly complex.

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## Riassunto

### *Efficacia delle salviette al perossido di idrogeno per la disinfezione delle superfici nelle strutture sanitarie*

**Introduzione.** Il metodo corretto di disinfezione delle superfici negli ospedali è uno strumento essenziale nella lotta alla diffusione delle infezioni nosocomiali causate da microrganismi multiresistenti. Attualmente, in commercio sono disponibili numerosi disinfettanti che possono essere utilizzati contro diversi microrganismi. Tuttavia, l'efficacia delle diverse molecole attive è controversa in letteratura.

**Disegno dello studio.** Lo scopo di questo studio è stato quello di valutare l'efficacia delle salviette a base di perossido di idrogeno (1.0 %) e tensioattivi di origine vegetale altamente specifici, contenuti nei prodotti H<sub>2</sub>O<sub>2</sub><sup>TM</sup> (Hi-speed H<sub>2</sub>O<sub>2</sub><sup>TM</sup>), contro alcuni microrganismi ospedalieri.

**Metodi.** L'efficacia delle salviette è stata testata contro ceppi nosocomiali e di controllo di *Staphylococcus aureus* resistente alla meticillina, *Pseudomonas aeruginosa* resistente ai carbapenemi, *Klebsiella pneumoniae* carbapenemasi, *Aspergillus fumigatus* e *Candida parapsilosis*. Nello specifico, l'attività in vitro è stata valutata utilizzando tre diverse tecniche: test su superficie di acciaio inossidabile, test di diffusione superficiale e test di diffusione in pozzetto.

**Risultati.** I tre diversi metodi testati confermano la buona efficacia delle salviette contro i più comuni batteri multiresistenti e contro i funghi.

**Conclusioni.** Questi dati mostrano che le salviette testate potrebbero essere un valido complemento al processo di disinfezione e potrebbero aiutare nella prevenzione delle infezioni correlate all'assistenza sanitaria.

## References

- Weber DJ, Kanamori H, Rutala WA. 'No touch' technologies for environmental decontamination: focus on ultraviolet devices and hydrogen peroxide systems. *Curr Opin Infect Dis*. 2016 Aug; **29**(4): 424-31. doi: 10.1097/QCO.000000000000284. PMID: 27257798.
- European Centre for Disease Prevention and Control (ECDC). Available from: <https://www.ecdc.europa.eu/en/healthcare-associated-infections> [Last accessed: 2024 Mar 1].
- Suleyman G, Alangaden G, Bardossy AC. The role of environmental contamination in the transmission of nosocomial pathogens and healthcare-associated infections. *Curr Infect Dis Rep*. 2018 Apr 27; **20**(6): 12. doi: 10.1007/s11908-018-0620-2. PMID: 29704133.
- Wißmann JE, Kirchhoff L, Brüggemann Y, Todt D, Steinmann J, Steinmann E. Persistence of Pathogens on Inanimate Surfaces: A Narrative Review. *Microorganisms*. 2021 Feb 9; **9**(2): 343. doi: 10.3390/microorganisms9020343. PMID: 33572303.
- Katzenberger RH, Rösel A, Vonberg RP. Bacterial survival on inanimate surfaces: a field study. *BMC Res Notes*. 2021 Mar 15; **14**(1): 97. doi: 10.1186/s13104-021-05492-0. PMID: 33722269.
- Lai Q, Spoletni G, Bianco G, Graceffa D, Agnes S, Rossi M, et al. SARS-CoV2 and immunosuppression: A double-edged sword. *Transpl Infect Dis*. 2020 Dec; **22**(6): e13404. doi: 10.1111/tid.13404. Epub 2020 Jul 17. PMID: 32639598.
- Vonberg RP, Weitzel-Kage D, Behnke M, Gastmeier P. Worldwide Outbreak Database: the largest collection of nosocomial outbreaks. *Infection*. 2011 Feb; **39**(1): 29–34. doi: 10.1007/s15010-010-0064-6. Epub 2010 Dec 3. PMID: 21153042.
- Song X, Vossebein L, Zille A. Efficacy of disinfectant-impregnated wipes used for surface disinfection in hospitals: a review. *Antimicrob Resist Infect Control*. 2019 Aug 19; **8**: 139. doi: 10.1186/s13756-019-0595-2. PMID: 31452873.
- Facciola A, Pellicanò GF, Visalli G, Paolucci IA, Venanzi Rullo E, Ceccarelli M, et al. The role of the hospital environment in the healthcare-associated infections: a general review of the literature. *Eur Rev Med Pharmacol Sci*. 2019 Feb; **23**(3): 1266-1278. doi: 10.26355/eurrev\_201902\_17020. PMID: 30779096.
- Squeri R, Genovese C, Palamara MA, Trimarchi G, La Fauci V. "Clean care is safer care": correct handwashing in the prevention of healthcare associated infections. *Ann Ig*. 2016 Nov-Dec; **28**(6): 409-415. doi: 10.7416/ai.2016.2123. PMID: 27845475.
- Paleckyte A, Dissanayake O, Mpagama S, Lipman MC, McHugh TD. Reducing the risk of tuberculosis transmission for HCWs in high incidence settings. *Antimicrob Resist Infect Control*. 2021 Jul 19; **10**(1): 106. doi: 10.1186/s13756-021-00975-y. PMID: 34281623.
- Boyce JM. Modern technologies for improving cleaning and disinfection of environmental surfaces in hospitals. *Antimicrob Resist Infect Control*. 2016 Apr 11; **5**(10): 10. doi: 10.1186/s13756-016-0111-x. PMID: 27069623.
- Diella G, Caggiano G, Apollonio F, Triggiano F, Stefanizzi P, Fasano F, et al. SARS-CoV-2 RNA viability on high-touch surfaces and evaluation of a continuous-flow ozonation treatment. *Ann Ig*. 2023 Jan-Feb; **35**(1): 112-120. doi: 10.7416/ai.2022.2547. Epub 2022 Oct 12. PMID: 36222607.

14. Triggiano F, Caggiano G, Lopuzzo M, Diella G, Apollonio F, Fasano F, et al. No-Touch Automated Disinfection System Based on Hydrogen Peroxide and Ethyl Alcohol Aerosols for Use in Healthcare Environments. *Int J Environ Res Public Health*. 2022 Apr 17; **19**(8): 4868. doi: 10.3390/ijerph19084868. PMID: 35457735.
15. Caggiano G, Apollonio F, Triggiano F, Diella G, Stefanizzi P, Lopuzzo M, D'Ambrosio M, Bartolomeo N, Barbuti G, Sorrenti GT, et al. SARS-CoV-2 and Public Transport in Italy. *Int J Environ Res Public Health*. 2021 Oct 29; **18**(21): 11415. doi: 10.3390/ijerph18179404. PMID: 34769932.
16. Caggiano G, Triggiano F, Apollonio F, Diella G, Lopuzzo M, D'Ambrosio M, et al. SARS-CoV-2 RNA and Supermarket Surfaces: A Real or Presumed Threat? *Int J Environ Res Public Health*. 2021 Sep 6; **18**(17): 9404. doi: 10.3390/ijerph18179404. PMID: 34501993.
17. Gidari A, Sabbatini S, Bastianelli S, Pierucci S, Busti C, Bartolini D, et al. SARS-CoV-2 Survival on Surfaces and the Effect of UV-C Light. *Viruses*. 2021 Mar 5; **13**(3): 408. doi: 10.3390/v13030408. PMID: 33807521.
18. Zhou X, Fu H, Du G, Wei X, Zhang B, Zhao T. SARS-CoV-2 RNA detection on environmental surfaces in COVID-19 wards. *PLoS One*. 2023 May 25; **18**(5): e0286121. doi: 10.1371/journal.pone.0286121. PMID: 37228102.
19. Marcenac P, Park GW, Duca LM, Lewis NM, Dietrich EA, Barclay L, et al. Detection of SARS-CoV-2 on Surfaces in Households of Persons with COVID-19. *Int J Environ Res Public Health*. 2021 Aug 2; **18**(15): 8184. doi: 10.3390/ijerph18158184. PMID: 34360477.
20. Cherrie JW, Cherrie MPC, Smith A, Holmes D, Semple S, Steidle S, et al. Contamination of Air and Surfaces in Workplaces with SARS-CoV-2 Virus: A Systematic Review. *Ann Work Expo Health*. 2021 Oct 9; **65**(8): 879-892. doi: 10.1093/annweh/wxab026. PMID: 34329379.
21. Bae SH, Shin H, Koo HY, Lee SW, Yang JM, Yon DK. Asymptomatic transmission of SARS-CoV-2 on evacuation flight. *Emerg Infect Dis*. 2020 Nov; **26**(11): 2705-2708. doi: 10.3201/eid2611.203353. Epub 2020 Aug 21. PMID: 32822289.
22. Cai J, Sun W, Huang J, Gamber M, Wu J, He G. Indirect virus transmission in cluster of COVID-19 cases, Wenzhou, China, 2020. *Emerging Infect Dis*. 2020 Jun; **26**(6): 1343. doi: 10.3201/eid2606.200412. Epub 2020 Jun 17. PMID: 32163030.
23. Xie C, Zhao H, Li K, Zhang Z, Lu X, Peng H, et al. The evidence of indirect transmission of SARS-CoV-2 reported in Guangzhou, China. *BMC Public Health*. 2020 Aug 5; **20**(1): 1202. doi: 10.1186/s12889-020-09296-y. PMID: 32758198.
24. Boyce JM. A review of wipes used to disinfect hard surfaces in health care facilities. *Am J Infect Control*. 2021 Jan; **49**(1): 104-114. doi: 10.1016/j.ajic.2020.06.183. Epub 2020 Jun 19. PMID: 32569612.
25. Weber DJ, Anderson D, Rutala WA. The role of the surface environment in healthcare-associated infections. *Curr Opin Infect Dis*. 2013 Aug; **26**(4): 338-344. doi: 10.1097/QCO.0b013e3283630f04. PMID: 23743816.
26. Block SS. Peroxygen compounds. In: Block SS, ed. *Disinfection, sterilization, and preservation*. Philadelphia: Lippincott Williams & Wilkins; 2001: 185-204. doi:10.1017/S0195941700084289.
27. Sattar SA, Springthorpe VS, Rochon M. A product based on accelerated and stabilized hydrogen peroxide: Evidence for broad-spectrum germicidal activity. *Canadian J Infect Control*. 1998; **33**: 123-130.
28. Linley E, Denyer SP, McDonnell G, Simons C, Maillard JY. Use of hydrogen peroxide as a biocide: new consideration of its mechanisms of biocidal action. *J Antimicrob Chemother*. 2012 Jul; **67**(7): 1589-96. doi: 10.1093/jac/dks129. Epub 2012 Apr 24. PMID: 22532463.
29. Sies H. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox Biol*. 2017 Apr; **11**: 613-619. doi: 10.1016/j.redox.2016.12.035. Epub 2017 Jan 5. PMID: 28110218.
30. Bharti B, Li H, Ren Z, Zhu R, Zhu Z. Recent advances in sterilization and disinfection technology: A review. *Chemosphere*. 2022 Dec; **308**(Pt 3): 136404. doi: 10.1016/j.chemosphere.2022.136404. Epub 2022 Sep 19. PMID: 36165840.
31. UNI EN ISO 4833-1:2013. Horizontal Method for the Enumeration of Microorganisms. Part 1: Colony Count at 30 Degrees C by the Pour Plate Technique. International Organization for Standardization. Milan, Italy; 2013.
32. Microbiology of Food and Animal Feeding Stuffs-Enumeration of Yeasts and Moulds by Colony-Count Technique at 25 °C-Routine Method. Association Francaise de Normalisation. La Plaine Saint-Denis Cedex, France; 2013.
33. Montagna MT, Triggiano F, Barbuti G, Bartolomeo N, De Giglio O, Diella G, et al. Study on the *In Vitro* Activity of Five Disinfectants against Nosocomial Bacteria. *Int J Environ Res Public Health*. 2019 May 29; **16**(11): 1895. doi: 10.3390/ijerph16111895. PMID: 31146343.
34. Saha O, Rakhi NN, Istiaq A, Islam I, Sultana M, Hossain MA, et al. Evaluation of Commercial Disinfectants against *Staphylococcus lentus* and *Micrococcus* spp. of Poultry Origin. *Vet Med Int*. 2020 Sep 25; 2020: 8811540. doi: 10.1155/2020/8811540. PMID: 33062244.
35. Athanassiadis B, Abbott PV, George N, Walsh LJ. An in vitro study of the antimicrobial activity of some endodontic medicaments and their bases using an agar well diffusion assay. *Aust Dent J*. 2009 Jun; **54**(2): 141-146. doi: 10.1111/j.1834-7819.2009.01107.x. PMID: 19473156.
36. Dancer SJ. Hospital cleaning in the 21st century. *Euro J Clin Microbiol Infect Dis*. 2011 Dec; **30**(12): 1473-1481. doi: 10.1007/s10096-011-1250-x. Epub 2011 Apr 17. PMID: 21499954.
37. Dettenkofer M, Wenzler S, Amthor S, Antes G, Motschall E, Daschner FD. Does disinfection of environmental surfaces influence nosocomial infection rates? A systematic review. *Am J Infect Control*. 2004 Apr; **32**(2): 84-89. doi: 10.1016/j.ajic.2003.07.006. PMID: 15057199.
38. Warren BG, Barrett A, Graves A, King C, Turner NA, Anderson D.J. An Enhanced Strategy for Daily Disinfection in Acute Care Hospital Rooms: A Randomized Clinical

- Trial. *JAMA Netw Open*. 2022 Nov 1; **5**(11): e2242131. doi: 10.1001/jamanetworkopen.2022.42131. PMID: 36378308.
39. Protano C, Cammalleri V, Romano Spica V, Valeriani F, Vitali M. Hospital environment as a reservoir for cross transmission: cleaning and disinfection procedures. *Ann Ig*. 2019 Sep-Oct; **31**(5): 436-448. doi: 10.7416/ai.2019.2305. PMID: 31304524.
  40. Rutala WA, Weber DJ. Surface disinfection: should we do it? *J Hosp Infect* 2001 Aug; **48** (Suppl A): S64-S68. doi: 10.1016/s0195-6701(01)90017-9. PMID: 11759030.
  41. Wu YL, Yang XY, Ding XX, Li RJ, Pan MS, Zhao X, et al. Exposure to infected/colonized roommates and prior room occupants increases the risks of healthcare-associated infections with the same organism. *J Hosp Infect*. 2019 Feb; **101**(2): 231-239. doi: 10.1016/j.jhin.2018.10.014. Epub 2018 Oct 22. PMID: 30359649.
  42. Cohen B, Cohen CC, Løyland B, Larson EL. Transmission of health care-associated infections from roommates and prior room occupants: A systematic review. *Clin Epidemiol*. 2017 May 23; **9**: 297-310 doi: 10.2147/CLEP.S124382. PMID: 28579835.
  43. Christenson EC, Cronk R, Atkinson H, Bhatt A, Berdiel E, Cawley M, et al. Evidence Map and Systematic Review of Disinfection Efficacy on Environmental Surfaces in Healthcare Facilities. *Int J Environ Res Public Health*. 2021 Oct; **18**(21): 11100. doi: 10.3390/ijerph182111100.
  44. Nseir S, Blazejewski C, Lubret R, Wallet F, Courcol R, Durocher A. Risk of acquiring multidrug-resistant Gram-negative bacilli from prior room occupants in the intensive care unit. *Clin Microbiol Infect*. 2011 Aug; **17**(8): 1201-1208. doi: 10.1111/j.1469-0691.2010.03420.x. Epub 2010 Dec 3.
  45. Torabi S, Zahra F. Disinfectants. 2022. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan.
  46. Rutala WA, Kanamori H, Gergen MF, Knelson LP, Sickbert-Bennett EE, Chen LF, et al; CDC Prevention Epicenters Program. Enhanced disinfection leads to reduction of microbial contamination and a decrease in patient colonization and infection. *Infect Control Hosp Epidemiol*. 2018 Sep; **39**(9): 1118-1121. doi: 10.1017/ice.2018.165. Epub 2018 Jul 31. PMID: 30060770.
  47. Qiu Y, Zhou Y, Chang Y, Liang X, Zhang H, Lin X, et al. The Effects of Ventilation, Humidity, and Temperature on Bacterial Growth and Bacterial Genera Distribution. *Int J Environ Res Public Health*. 2022 Nov 20; **19**(22): 15345. doi: 10.3390/ijerph192215345. PMID: 36430064.
  48. Wolkoff P. Indoor air humidity, air quality, and health—An overview. *Int J Hyg Environ Health*. 2018 Apr; **221**(3): 376-390. doi: 10.1016/j.ijheh.2018.01.015. Epub 2018 Jan 31. PMID: 29398406.
  49. Otter JA, Yezli S, French GL. The role played by contaminated surfaces in the transmission of nosocomial pathogens. *Infect Control Hosp Epidemiol*. 2011 Jul; **32**(7): 687-99. doi: 10.1086/660363. PMID: 21666400.
  50. Rutala WA, Weber DJ and Healthcare Infection Control Practices Advisory Committee (HICPAC). Guideline for disinfection and sterilization in healthcare facilities, 2008; last update: May 2019. Available from: <https://www.cdc.gov/infectioncontrol/pdf/guidelines/disinfection-guidelines-H.pdf> [Last accessed: 2024 Mar 1]. doi: 10.1086/650197.
  51. Triggiano F, Caggiano G, Lopuzzo M, Diella G, Apollonio F, Fasano F, et al. No-Touch Automated Disinfection System Based on Hydrogen Peroxide and Ethyl Alcohol Aerosols for Use in Healthcare Environments. *Int J Environ Res Public Health*. 2022 Apr 17; **19**(8): 4868. doi: 10.3390/ijerph19084868. PMID: 35457735.
  52. Otter JA, Yezli S, Perl TM, Barbut F, French GL. The role of ‘no-touch’ automated room disinfection systems in infection prevention and control. *J Hosp Infect*. 2013 Jan; **83**(1): 1-13. doi: 10.1016/j.jhin.2012.10.002. doi: 10.1016/j.jhin.2012.10.002. Epub 2012 Nov 26. PMID: 23195691.
  53. Ríos-Castillo AG, González-Rivas F, Rodríguez-Jerez JJ. Bactericidal Efficacy of Hydrogen Peroxide-Based Disinfectants Against Gram-Positive and Gram-Negative Bacteria on Stainless Steel Surfaces. *J Food Sci*. 2017 Oct; **82**(10): 2351-2356. doi: 10.1111/1750-3841.13790. Epub 2017 Aug 23. PMID: 28833105.
  54. Song X, Vossebein L, Zille A. Efficacy of disinfectant-impregnated wipes used for surface disinfection in hospitals: a review. *Antimicrob Resist Infect Control*. 2019 Aug 19; **8**: 139. doi: 10.1186/s13756-019-0595-2. PMID: 31452873.
  55. Kelley AM, Voorn MG, Tembo GM, Horn CM, Li X, Teska PJ, et al. Contact time has limited impact on the efficacy of disinfectant towelettes when tested under conditions reflective of realistic use. *Antimicrob Resist Infect Control*. 2023 Jul 16; **12**(1): 71. doi: 10.1038/s41598-023-32876-y. PMID: 37455322.
  56. Edwards NWM, Best EL, Connell SD, Goswami P, Carr CM, Wilcox MH, et al. Role of surface energy and nano-roughness in the removal efficiency of bacterial contamination by nonwoven wipes from frequently touched surfaces. *Sci Technol Adv Mater*. 2017 Mar 14; **18**(1): 197-209. doi: 10.1080/14686996.2017.1288543. PMID: 28469734.
  57. Moccia G, Motta O, Pironti C, Proto A, Capunzo M, De Caro F. An alternative approach for the decontamination of hospital settings. *J Infect Public Health*. 2020 Dec; **13**(12): 2038-2044. doi: 10.1016/j.jiph.2020.09.020. Epub 2020 Oct 21. PMID: 33289645.
  58. D’Accolti M, Soffritti I, Bonfante F, Ricciardi W, Mazzacane S, Caselli E. Potential of an Eco-Sustainable Probiotic-Cleaning Formulation in Reducing Infectivity of Enveloped Viruses. *Viruses*. 2021 Nov 4; **13**(11): 2227. doi: 10.3390/v13112227. PMID: 34835033.