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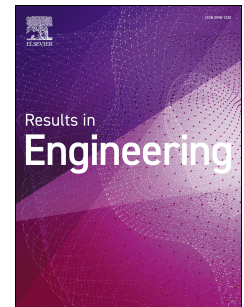
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Long-lasting multi-surface disinfectant: evaluation of efficiency and durability

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ABSTRACT

The present work aimed to study the efficacy and durability of a long-term multi-surface disinfectant. The disinfectant formulation under study had hydrogen peroxide and ethanol as active substances, among other substances. The antimicrobial activity of the formulation components and their stability on porous, non-porous, and everyday surfaces was evaluated. The stability test result shows that the disinfectant still has antimicrobial activity after 2 years of storage, even with the loss of 80 % of one of the active substances. The results show that the disinfectant has an impressive antimicrobial activity for porous surfaces and good performance for non-porous surfaces. The analysis of antimicrobial activity on everyday surfaces shows total fungal inhibition and a notable decrease in bacteria colonization after 7 days of disinfectant application.

KEYWORDS

Disinfectant; hydrogen peroxide; ethanol; antimicrobial activity

1. Introduction

The hygiene needs and customs have changed over the years by society's imposition, due to sociodemographic characteristics and hygiene habits [1,2]. These needs were even more accentuated with the appearance of the pandemic caused by SARS-Cov-2 [2]. During the pandemic, chemical disinfectants were ubiquitously and routinely used in community environments, especially on common touchable surfaces in public settings, as a means of controlling the virus spread [3].

The pandemic has spurred an increase in demand for cleaning products and disinfectants due to the growing concerns about hygiene, health, and safety, as of March 2021, there were 535 EPA-approved chemical disinfectant products for COVID-19 disinfection [2,3]. In addition, the growth of the disinfectant market is also attributed to the increase in the appearance of infections acquired in the hospital environment and the implementation of regulations favourable to the use of surface disinfectants, both in the healthcare industry, but also in other sectors, such as the food industry [2].

The term disinfection is defined as a process of the antimicrobial reduction of the number of viable microorganisms, it refers to killing or inactivation of them to a level previously specified as appropriate for their intended further handling or use [4–6].

Disinfectants are widely acknowledged for removing microorganisms from the surface of the objects and transmission media, can effectively control infectious diseases by inhibiting or destroying the pathogenic growth, including bacteria and virus [7,8].

The disinfection process plays a vital role in ecological health and safety of life and can be used in a wide variety of applications, including surfaces, devices, liquids and products, foods, in medical, water treatment and distribution, food processing, agricultural industry, health care settings, and other fields [4,7]. The process of disinfection usually involves chemicals, heat, irradiation or UV radiation [5,9].

Surfaces have porous or non-porous properties that change the performance and the way in which the disinfectant works. A porous surface can be defined as a surface with pores of various shapes and sizes, that is, space not occupied by the main structure of atoms that constitute the surface structure [10,11]. The most important characteristics of porous materials are surface area and pore size [12]. There are several characteristics to classify porous materials, depending on their pore size, physical properties, composition, synthesis routes, function, etc. [12]. A non-porous surface is a surface that does not contain pores, that is, it corresponds to a compact solid, to a macroscopic point of view. [13–15]. Nonporous surface a material that lacks minute openings or crevices that keep air, water and bacteria from entering the item, such as metal, glass, silicone, and plastic [13–15].

The disinfectant and other chemical preparations and all substances considered as active principals are regulated by an entity called European Chemical Agency (ECHA), which promotes the application of European Union legislation referent to a chemical product to benefit human health resources, and the environment, never forgetting the innovation and competitiveness [16].

For COVID-19, the most used disinfecting agents are quaternary ammonium compounds, hydrogen peroxide, sodium hypochlorite and ethanol, which account for two-thirds of the active ingredients in current EPA-approved disinfectant products for the

novel coronavirus [3]. Nearly 81% of the disinfectant products use only one biocidal agent, while the rest of them use two or more in their formulation [3]. Since most of these commercially available disinfectants have a very short period of action, it is imperative to renew their application at short intervals[16,17].

The present study intends to show a new concept of disinfectant *Care Us* (developed by Success Gadget, not yet commercialized), with prolonged antimicrobial action. This study appears to perform tests for the first time to evaluate the antimicrobial activity of *Care Us* on porous and non-porous surfaces and on everyday surfaces. This consists of a disinfectant with two biocidal agents that proposes to have greater durability (7 days), a novelty in the disinfection allegations.

2. Material and methods

2.1. *Care Us* Production

The disinfectant developed by the company *Success Gadget* is named *Care Us* consists in a disinfectant that was developed to be applied to various surfaces with distinctive characteristics and to have a lasting antimicrobial effect, for periods of time greater than 72 h of current disinfectant. The product is a compound of two active substances, in this case, hydrogen peroxide and ethanol, and excipients, such as emulsifiers, cleaning agents, fixator, and Sil2U[®] particles. The product was created by the company in order to have a long-lasting antimicrobial activity and high applicability on different surfaces with different characteristics.

2.2. Sample Preparation

In this study two types of textile samples (Fig. 1) were tested as porous surface, supplied by a local textile industry (*Malhas Sonix, S.A., Barcelos, Portugal*). The sample I is a single jersey with 137 g/m², 30/1, 100% cotton, BCI (Better Cotton Initiative), set

28, with optical brightener. The sample II is an American terry 306 g/m², 30/1, 100% cotton, BCI, set 20, dyed with reactive dyes, with trichromy technique. To each assay, 6 samples were used for control and 6 samples for each time of application of *Care Us* (3 samples referring to the 0 h incubation time and the remaining 3 samples for the 24 h incubation time), these were cut to obtain a mass of (0.40 ± 0.05) g. All samples were sterilized in an autoclave at 121 °C for 15 min.

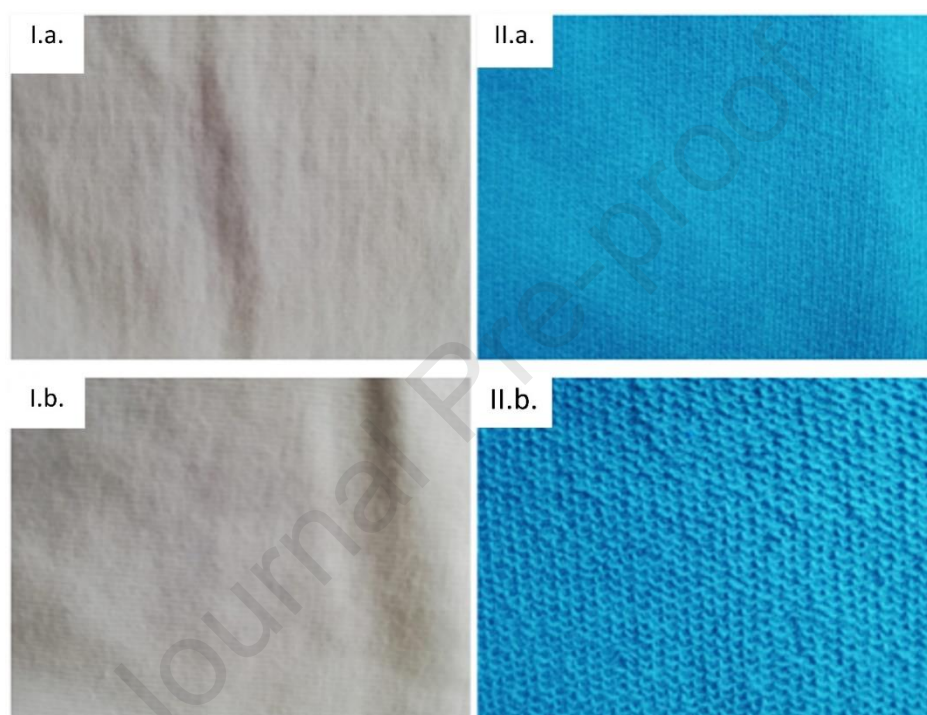


Fig. 1- Textile samples used in antimicrobial activity tests; sample I – low porosity and sample II – high porosity (a. front; b. reverse)

Aluminium samples (Fig. 2), provided by Correia & Cardoso, Barcelos, Portugal, were also tested as non-porous surface. The samples under study had a size of 50 mm × 50 mm. To each assay, 6 samples were used for control and 3 samples for each time of application of *Care Us* (3 samples referring to the zero incubation time and the remaining 3 samples for the 24 h incubation time).

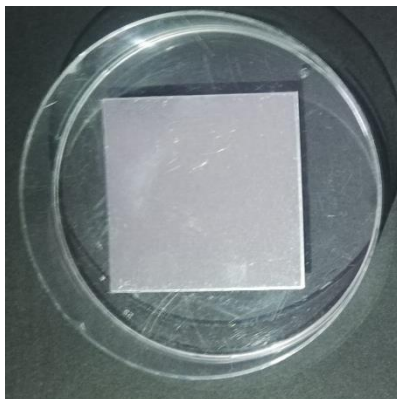


Fig. 2 - Aluminium samples used in antimicrobial activity tests.

Aluminium samples were sterilized by exposing both sides to UV light for 30 min.

2.3. Quantification of the Active Reagent - Hydrogen Peroxide

2.3.1. Titration of the liquid medium by iodometry

Following the IS:7045-1973 standard, the hydrogen peroxide present in the formulation of *Care Us*, the disinfectant under study, was quantified. For that, 10 g of the formulation were added to 50 mL of distilled water, 10 mL of sulfuric acid (H_2SO_4) at 20% (v/v), and 10 mL of 1% (w/v) potassium iodide (KI), in addition to 2 drops of ammonium molybdate, catalyst, were added and kept under magnetic agitation. Then, the mixture was titrated with sodium thiosulfate ($Na_2S_2O_3$) at 0.1 N, recording the volume necessary for a colour change from brown to colourless, and this change was kept for at least 30 s. The percentage of hydrogen peroxide was calculated based on equation 1 [18].

$$\% H_2O_2 = (V_{Na_2S_2O_3} \times 0.1 \times 1.7) / m \quad (1)$$

Where, $V_{Na_2S_2O_3}$ is the $Na_2S_2O_3$ volume titrated, and m is the mass of the sample.

2.3.2. Titration of textile samples by iodometry

The test of quantification of the hydrogen peroxide present in the textile samples was carried out with the aim of determining the losses of active reagent that occur over the time of the study.

Textile samples with a mass of (0.40 ± 0.05) g were used to which 8 mL of distilled water and 0,8 mL of sulfuric acid (H_2SO_4) at 20% (v/v) were added. With the aid of a glass rod, it was stirred and left to rest for 10 min. In the next step, 0.8 mL of a 1 % (w/v) potassium iodide (KI) was added, the mixture was shaken again and placed in the dark for 20 min. After, 1 drop of ammonium molybdate, catalyst, was added, placing the solution under magnetic agitation. Then, the sample was titrated with sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) at 0.1 N, recording the volume necessary for a colour change from brown to colourless, and this change was kept for at least 30 s. The percentage of hydrogen peroxide was calculated based on equation 1 [18].

2.4. Accelerated stability test

The accelerated stability test had as main aim to supply useful guidelines on the performance of the product, therefore the formulation is placed in an oven at (54 ± 2) °C for 14 days, which can be equivalent of 2 years of shelf stability [19].

For this test, about 40 mL of the formulation were placed in six 120 mL diagnostic containers and were subjected to a temperature of (54 ± 2) °C for 14 days in an oven, and the times points of day 0 (before placement in the oven), day 7, and day 14 were analysed. For each time there were two containers, one to analyse by the titration of the liquid medium by iodometry and other to determine the density and pH of the sample.

The measurement of the pH of the formulation was performed using a Hannah Instruments Edge® pH meter – HI2020.

The density of the formulation was determined using a pycnometer. The value of density (ρ) was calculated based on equation 2.

$$\rho = (m_{fp} - m_{ep})/V_p \quad (2)$$

Where, m_{fp} is full pycnometer mass, m_{ep} is empty pycnometer mass and V_p is pycnometer volume.

2.5. Wettability of textile samples

The wettability behaviour was assessed by the sessile drop contact angle technique, using an automated contact angle measurement apparatus (OCA 20 Plus; Dataphysics, Germany). The water contact angle (WCA) was measured using a 500 μ L syringe with ultrapure water (Millipore, 18.3 M Ω :CM) at room temperature.

This test was carried out to prove the different absorption behaviour of the inoculum between sample I and sample II, which were used for antimicrobial activity tests.

2.6. Antimicrobial evaluation

This study aimed to evaluate the antimicrobial activity of *Care Us* for different application times, either on porous and non-porous materials and, finally, on everyday surfaces.

To evaluate the antimicrobial activity in porous materials, in textiles, the ISO 20743:2007 standard was followed and adapted for the yeast. For non-porous materials, in this case aluminium substrates, the ISO 22196:2011 standard was followed and adapted for the yeast. The antimicrobial activity tests performed on everyday surfaces were carried out according to ISO 18593:2004.

2.6.1. Microorganisms and growth conditions

The antibacterial activity of *Care Us* was tested against three bacteria *Staphylococcus aureus* ATCC 6538, *Klebsiella pneumoniae* ATCC 11296 and *Escherichia coli* CECT 434. Each bacterium was inoculated in 30 ml of Tryptic Soy Broth (TSB) and incubated for 18 h at 37 °C, with a rotation frequency of 120 min⁻¹. The choice

of these three bacteria was based on the standards selected. The antifungal activity of *Care Us* was tested against *Candida albicans* SC5314. The yeast was inoculated in 30 ml of Sabouraud Dextrose Broth (SDB) and incubated for 18 h at 37 °C, with a rotation frequency of 120 min⁻¹.

2.6.2. Evaluation of antimicrobial activity of the disinfectant understudy

The antimicrobial activity of *Care Us* was evaluated by disk agar diffusion test. For that, the disinfectant from the accelerated stability test was used and evaluated at once after application (0 days) and after 7 and 14 days of testing.

After the incubation period, the inoculum was adjusted to an optical density (OD) below 1.0 at 620 nm and properly diluted to 1×10^8 CFU/mL for bacteria, for the yeast, the cell density was adjusted to 1×10^8 cells/mL, using a Neubauer haemocytometer (Marienfeld, Lauda-Königshofen, Germany). An aliquot of cellular suspension (100 µL) was spread in Tryptic Soy Agar (TSA) and Sabouraud Dextrose Agar (SDA) petri dishes, to bacteria and yeast, respectively. After medium solidification, the blank disks with 20 µL of the disinfectant from the accelerated stability test (0, 7 and 14 days) were placed on the top of the agar plate, incubated for 24 h at 37 °C. After the incubation period, the halo inhibition zone (zone of transparent medium, which means that there is no bacteria growth) formed around the sample was photographed to record the results (images captured with Image Lab™ software).

2.6.3. Evaluation of antimicrobial activity on porous surfaces

The antimicrobial activity on porous surfaces was evaluated following the standard ISO 20743:2007. After the incubation period, the inoculum was adjusted to an OD below 1.0 at 620 nm and properly diluted to 2.5×10^5 CFU/mL for bacteria, for the yeast, the cell density was adjusted to 2.5×10^5 cells/mL, using a Neubauer haemocytometer (Marienfeld, Lauda-Königshofen, Germany). Each textile sample was

previously sprayed with 1 pump (weight $\sim 0,11$ g) of *Care Us* and after 2, 24, 48 and 168 h (7 days), was inserted in diagnostic containers and inoculated with 200 μ L of cell's suspension. Then the diagnostic containers were incubated at $(37 \pm 2) ^\circ\text{C}$ for 24 h [20].

For 0 h control samples, the procedure described below was used, without incubation period. After the incubation period, 20 mL of TSB or SDB solution with polysorbate 80 (PanReac AppliChem) were added and then shaken for 20 s, for bacteria or yeast, respectively. After, the bacteria were incubated with serial dilutions on TSA plates at $(37 \pm 2) ^\circ\text{C}$ for 24 h and then the number of Colony Forming Units (CFU) was counted. For yeast, the same procedure was followed, replacing the TSA plates by SDA. The value of antimicrobial activity was calculated based on equation 3 [20].

$$A = \lg C_t - \lg T_t \quad (3)$$

Where $\lg C_t$ is the average of the logarithm of base 10 of the number of microorganism, in CFU/mL, obtained from the three control textile samples after an incubation of 24 h and $\lg T_t$ is the average of the logarithm of base 10 of the number of microorganism, in CFU/mL, obtained from the three samples of textile with the different time of the disinfectant application of after an incubation of 24 h. In case of no colonies are counted, the logarithm for the colony will be considered one [20].

2.6.4. Evaluation of antimicrobial activity on non-porous surfaces

For non-porous surfaces, the antimicrobial activity was evaluated following the standard ISO 22196:2011. After the incubation period, the inoculum was adjusted to an OD below 1.0 at 620 nm and properly diluted to 6×10^5 CFU/mL for bacteria, for the yeast, the cell density was adjusted to 6×10^5 cells/mL, using a Neubauer haemocytometer (Marienfeld, Lauda-Königshofen, Germany). Each aluminium sample previously sprayed with 2 pumps of *Care Us* with the application time of 2, 24, 48 and 168 h, was placed in

Petri dishes and inoculated with 400 μL of the resultant cell's suspension. Then, the aluminium samples were covered with a polypropylene film of size 40 mm $\times$ 40 mm and pressed to ensure that the inoculum covered this entire area. The Petri dishes were covered and incubated at $(37 \pm 2)^\circ\text{C}$ for 24 h. [21]

For time 0 h control samples, the procedure described below was used, without incubation period. After the incubation period, 10 ml of TSB solution was added and shaken with a rotation frequency of 100 min^{-1} to homogenize, the samples surface was properly scraped to detach the cells attached. In the case of yeast, the TSB solution was replaced by SDB. The bacteria were incubated with serial dilutions on TSA plates at $(37 \pm 2)^\circ\text{C}$ for 24 h, and then the number of CFU was counted. For yeast, the same procedure was followed, replacing the TSA plates by SDA. The value of antimicrobial activity was calculated based on equation 4 [21].

$$R = U_t - A_t \quad (4)$$

Where U_t is the average of the logarithm of base 10 of the number of bacteria, in CFU/ml, obtained from the three control aluminium samples after an incubation of 24 h and A_t is the average of the logarithm of base 10 of the number of bacteria, in CFU/ml, obtained from the three samples of aluminium with the different time of the disinfectant application after an incubation of 24 h. In case of no colonies are counted, the logarithm for the colony will be considered one [21].

2.6.5. Evaluation of antimicrobial activity on everyday surfaces

To analyse the performance of *Care Us* on everyday surfaces, it was applied to shelves and desks used daily. The procedure for these tests was adapted from the ISO 18593:2004 standard.

Initially, five zones were defined, referring to a control and application times of 2, 24, 48 and 168 h, as shown in Fig. 3. These zones had a useful area of 100 cm², delimited by an area covered with tape-glue [22].

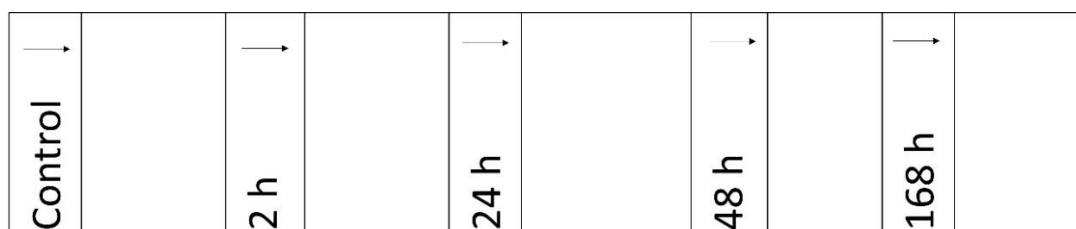


Fig. 3 - Scheme of the shelf used for testing antimicrobial activity on surfaces.

In the area referring to the control, no disinfectant was applied, while in the other areas it was applied according to the application times under study. This test was performed against native microorganisms residing on the test surface, no test organisms were seeded on the surface.

To collect the sample, a swab humidified in a tube about 2 mL of Phosphate Buffered Saline 1× (PBS 1×), was passed across the surface in parallel and then perpendicularly, always rotating on itself [22].

Finally, the swab was passed over a Petri dish containing TSA, inverted and incubated at (37 ± 2) °C for 24 h, for the appearance of bacteria. After this time, the plates were placed at room temperature for 168 h to analyse the emergence of fungi and yeasts [22].

2.7. Statistically Test

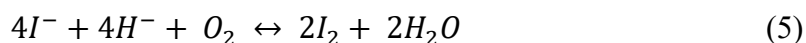
Data were expressed as the mean $\pm$ standard deviation (SD) of a least three independent experiments. All the results were statistically analysed using the GraphPad Prism 6 software. All tests were performed with a confidence level of 95%.

3. Results and discussion

3.1. Quantification of the Active Reagent - Hydrogen Peroxide

3.1.1. Titration of the liquid medium by iodometry

The theoretical value of hydrogen peroxide in the formulation is 1.49 % (m/m) and a value of (0.96 ± 0.02) % (m/m) has been experimentally obtained in the formulation. This means that 100 g of formulation contains 0.96 g of hydrogen peroxide. The differences between the theoretical value and the experimental value can be explained by a possible degradation of hydrogen peroxide in water and oxygen. The method used is extremely sensitive, since it depends on the investigator's decision of when the endpoint of the assay is, as it is a colorimetric method. Additionally, when using an iodide solution in an acidic medium, as is the case of the formulation under study, it is necessary to carry out the titration immediately, to avoid oxidation with atmospheric oxygen, as shown in Equation 5. The reaction rate increases to a lower pH, such as the formulation under study have a pH of 4.62 at 19 °C, which influences the oxidation of the iodide solution. Under neutral conditions, the reaction of iodide and oxygen ions is negligible, and the alkaline medium is completely impractical [23].



The concentration of the potassium iodide solution used is less than 4 % (m/v), which may potentiate losses by volatilization at room temperature [24]

3.1.2. Titration of textile samples by iodometry

Textile samples are representative samples of porous surfaces, which were used in antimicrobial activity tests and therefore were sprayed with the *Care Us*. In this sense, it is significant to quantify the hydrogen peroxide present in the samples and the loss rates that may occur over the test application times.

In this test, the objective was to relate time of application of the disinfectant (*Care Us*) with the percentage of hydrogen peroxide present in textile samples (Fig. 4).

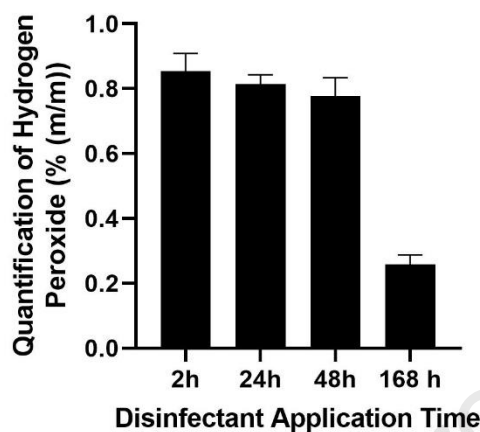


Fig. 4 - Quantification of hydrogen peroxide, by iodometry, as a function of disinfectant application time.

The quantification of hydrogen peroxide was theoretically calculated to assess whether the theoretical value is close to the experimental value. To obtain the theoretical value, the mass of the sample, the mass of the product applied, and the percentage of hydrogen peroxide placed in the formulation were used (Table 1).

Table 1. Theoretical quantification of hydrogen peroxide based on the values of textile mass and mass of *Care Us* sprayed on them.

Mass (g)		Quantification of Hydrogen Peroxide in 100 g of sample I (g)	
Textile	Spray <i>Care Us</i>	Theoretical	Experimental
0.40 ± 0.05	0.22 ± 0.03	0.85 ± 0.08	0.85 ± 0.06

Table 1 shows that the theoretical value coincides with the experimental value, which is an excellent indicator that the amount of hydrogen peroxide in the sample is the same as intended.

Comparing the times points between 2 and 24 h with 24 and 48 h, it is possible to see through Fig. 4, that the decrease in active substance is remarkably similar, being 4.7

% and 4.5 %, for the first and second time slots, respectively. As the time intervals are identical, it was also expected that the percentage of hydrogen peroxide reduction would also be close, as obtained.

For the longest time interval, from 48 to 168 h, higher decrease in hydrogen peroxide content is observed, corresponding to a loss of 66.7% of the active reagent. This is an expected result since over time the active reagent decomposes into water and oxygen, and this reaction is catalysed by the exposure to sunlight and hot temperatures as in everyday life.

3.2. Accelerated stability tests

In the accelerated stability test, *Care Us* was placed in an oven, subjected to a temperature of $(54 \pm 2) ^\circ\text{C}$, for 14 days the parameters under analysis were studied before placing the formulation in the oven, on the 7th day of the test and finally, on the 14th day, which corresponds to the end of the experiment. The results obtained are shown in Fig. 5.

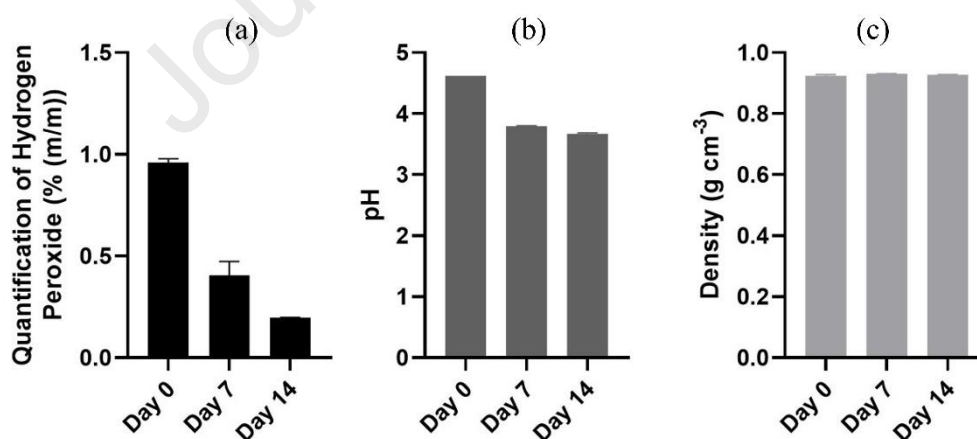


Fig. 5 - Factors analysed in the accelerated stability test for *Care Us* as a function of test time (a. quantification of hydrogen peroxide; b. pH; c. density).

The percentage of hydrogen peroxide at the end of the 14 days of testing is $(0.20 \pm 0.002) \% (m/m)$, as shown in Fig. 5 (a). The average content of the active substance was less than 80 % of the average content determined before storage of the formulation.

This loss of the active substance will have to be tackled by the addition of hydrogen peroxide stabilizers to ensure that the active substance remains in solution. This is a parameter that will continue to be studied, with the aim of improving the stability of the active substance and to ensure that losses are less than 10%, within the tolerance by ECHA [19,25].

The pH translates the values of the concentration of the hydrogen ion present in the solution to be analysed[26–28]. Temperature alters the dissociation equilibrium of water, easing the dissociation of oxygen and hydrogen bonds [26]. Increasing the temperature increases the activity of hydrogen ions, making the solution more acidic [28]. The pH readings are shown in Fig. 5 (b) and denote a tendency towards a reduction in pH. The stability of hydrogen peroxide is maximum for pH between 4.5 e 3.5, for a temperature of 20 °C, considering this information, the pH of the formulation is not out of adjustment with what would be ideal [25].

The density of formulation is the quotient between the mass of the formulation and its volume [29,30]. According to the density formula (eq. 2), it is inversely proportional to volume, the volume is a quantity that varies with temperature and pressure[29]. In the case of increasing the temperature, the molecules of the formulation will expand, increasing their volume and consequently decreasing the density [30,31]. The formulation was subjected to a temperature of (54 ± 2) °C and the readings were all taken at the same temperature 19 °C. The density parameter does not change during the accelerated stability test, as shown in Fig. 5 (c), thus, showing that the formulation does not undergo chemical changes.

3.3.Wettability behaviour of textile samples

The wettability of the textile samples to water (WCA) in room temperature is summarized in Fig. 6.

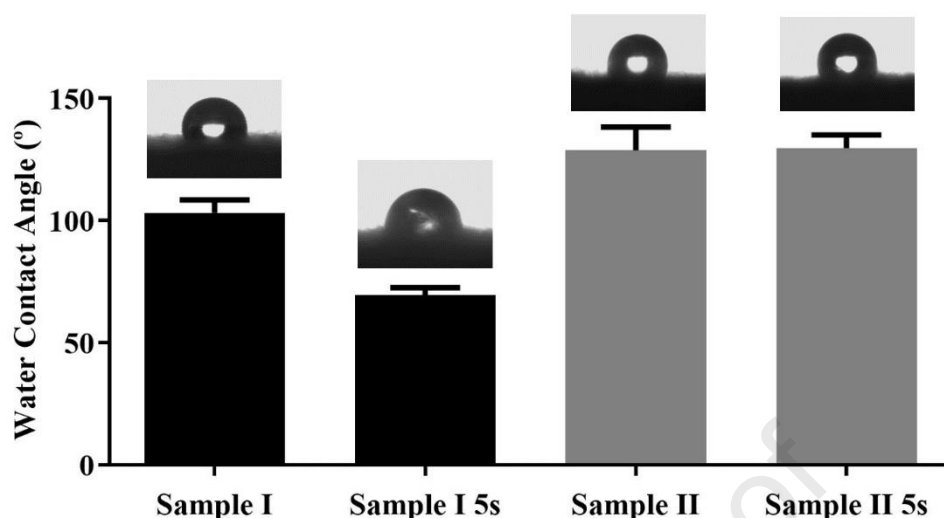


Fig. 6 - The water contact angle of the samples (I and II) measured immediately and 5s after the drop, represented as a mean $\pm$ standard deviation.

Surfaces can be classified as super-hydrophilic when, in the case of liquid water, $\theta \approx 0^\circ$, hydrophilic when $\theta < 90^\circ$ and hydrophobic when $\theta > 90^\circ$ [32]. The WCA measure immediately after the drop on the surface of the two samples shows a hydrophobic character, since $\theta > 90^\circ$, exhibited by both samples. After 5 s of the drop, the sample I shows a different behaviour, the θ values underwent a marked reduction to values below than 90° , suggesting a lower hydrophobic character contrary to sample II which continues with θ values higher than 90° , proving the permanence of its hydrophobic character. Multimedia video 1 shows that after just 30 s the drop disappear in sample I, being completely absorbed by the sample and video 2 shows that after 30 s the initial appearance of the drop remains.

3.4. Antimicrobial evaluation

3.4.1. Evaluation of antimicrobial activity of the disinfectant

The antimicrobial activity of *Care Us* before, in an intermediate time of 7 days of testing, and at the end of the accelerated stability test, 14 days of testing, is summarized in Fig. 7.

The results of agar diffusion tests present a certain inhibiting effect on the growth of *S. aureus*, *K. pneumoniae*, *E. coli* and *C. albicans*, since the growth of an inhibition halo is clear (transparent biological medium, with no bacteria growth) formed around the samples. The halo formed around the samples of *K. pneumoniae* is bigger than the other strains, proving to be the most susceptible microorganism to *Care Us*, the disinfectant under study.

The zone of inhibition for all cultures decreases as the accelerated stability test progresses, however the halos remain with considerable dimensions, showing that *Care Us* still has antimicrobial activity after 2 years of shelf stability, even with the loss of 80 % of one active substance.

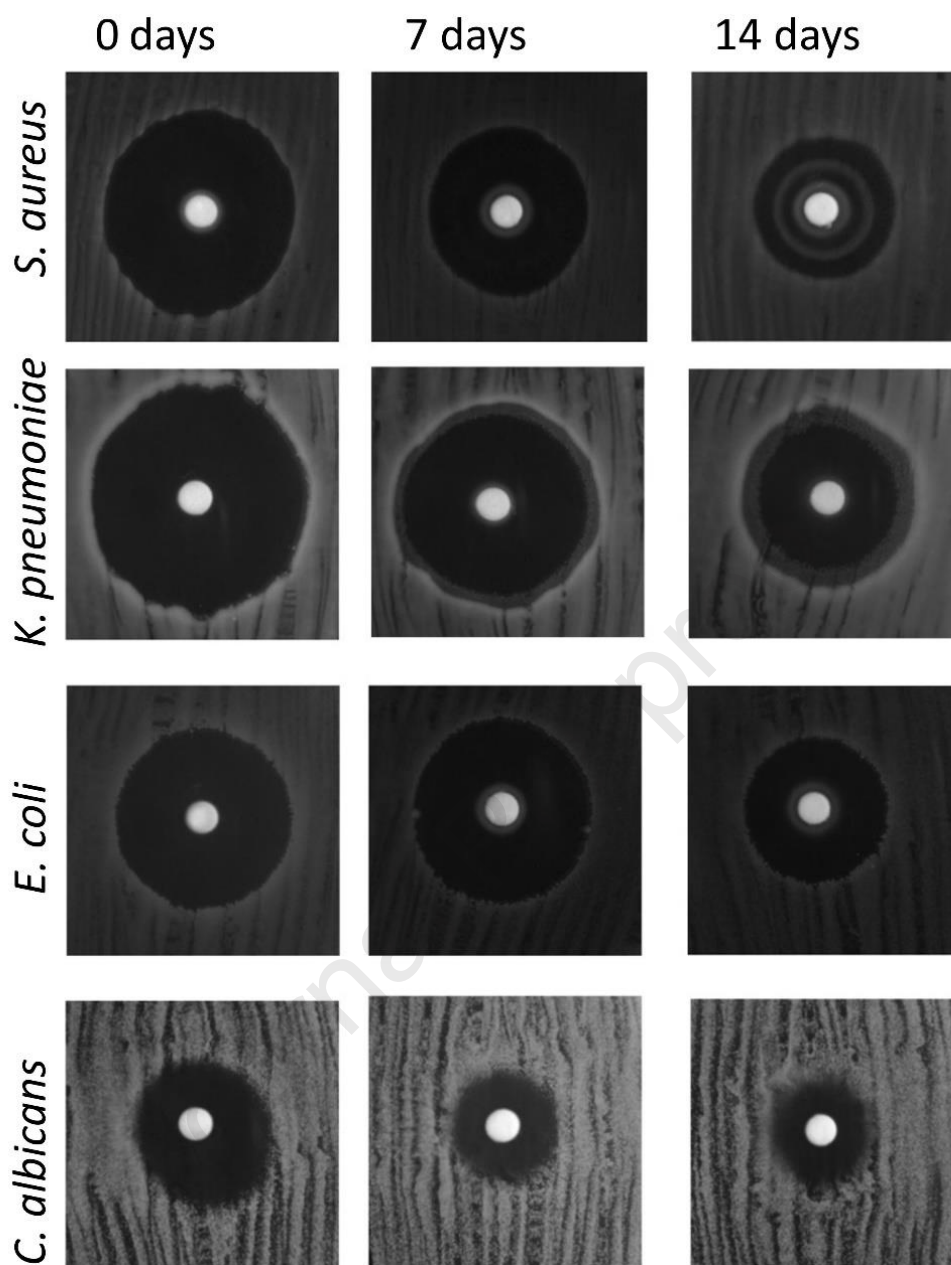


Fig. 7 - Agar disk diffusion test of the disinfectant from the accelerated stability test (0, 7 and 14 days).

3.4.2. Evaluation of antimicrobial activity on porous surfaces

After the application of the standard ISO 20743:2007, it was possible to build the graphs presents in Fig. 8, for sample I and Fig. 9, for sample II.

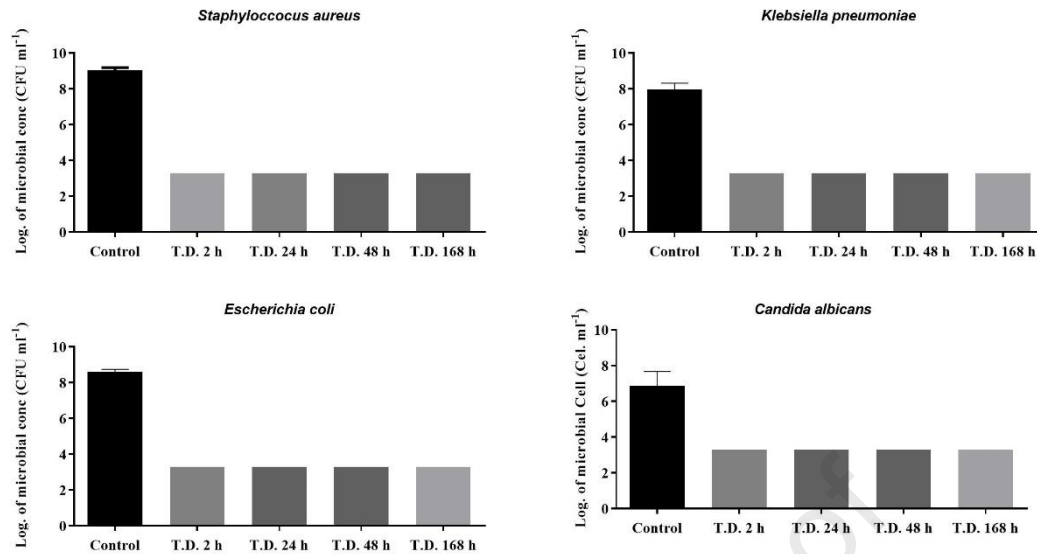


Fig. 8 - Microbial concentration logarithm after 24 h contact between sample I and microorganism, for different time of disinfectant application, samples do not show standard deviation since there was no microbial growth.

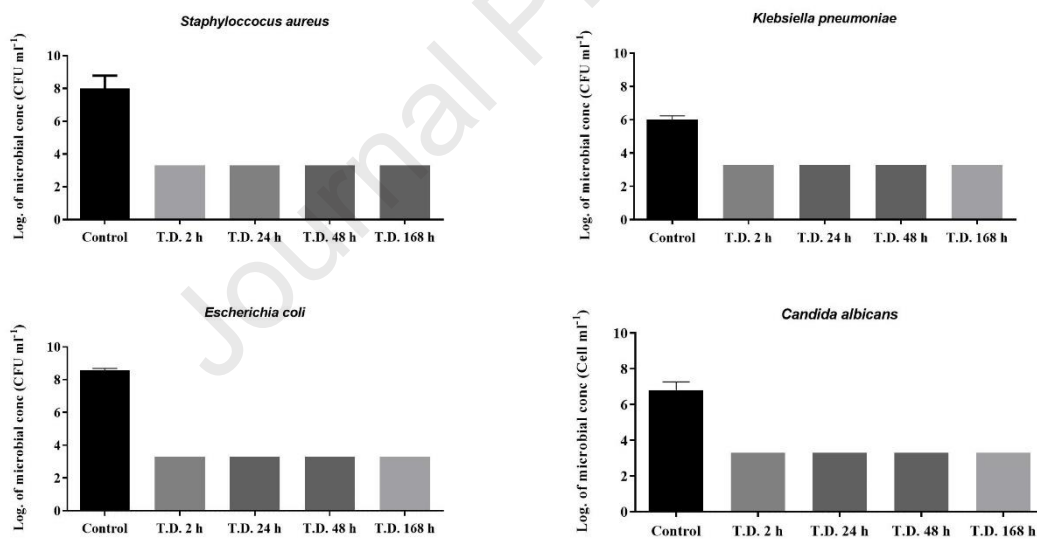


Fig. 9 - Microbial concentration logarithm after 24 h contact between sample II and microorganism, for different time of disinfectant application, samples do not show standard deviation since there was no microbial growth.

The first conclusion after analysing Figs. 8 and 9, is that *Care Us* has an antibacterial activity for 168 h for all bacteria under study and also has an antifungal activity for 168 h for *C. albicans*, since all values of antimicrobial activity are higher than

2 log (Table 2) and, according to the Japanese Industrial Standard Z 2801:2000, those values greater than 2 log means antimicrobial activity[33].

Table 2 - Antimicrobial activity for sample I and sample II, for *S. aureus*, *K. pneumoniae*, *E. coli* and *C. albicans*, for different time of disinfectant application

	Time Points (h)	Sample I				Sample II			
		2	24	48	168	2	24	48	168
Microorganism	<i>S. aureus</i>			> 5				> 4	
	<i>K. pneumoniae</i>			> 4				> 2	
	<i>E. coli</i>			> 5				>5	
	<i>C. albicans</i>			> 3				> 2	

As there was no microbial growth in the samples sprayed with *Care Us*, the differences between the values of the antimicrobial activity presented in Table 2 are due to the growth in the control samples, which was not the same for all bacteria, *K. pneumoniae*, showed less growth and consequently, the values referring to antimicrobial activity are lower.

In particular, sample I shows greater antimicrobial activity when compared to sample II, for the *S. aureus*, *K. pneumoniae* and *C. albicans* assays, sample I has a higher inoculum absorption capacity when compared to sample II. Video 1 shows complete absorption of water drop after 30 s for sample I while video 2 shows that sample II needs more time for water drop absorption. This fact leads to a greater ability allow the spread of the microbial inoculum.

The earlier fact is not verified in the *E. coli* tests since it has the same growth capacity in both sample I and sample II. Both *E. coli* and *K. pneumoniae* have a cell wall composed of polysaccharides and these help adhesion to the more hydrophobic textile

substrate [34]. Therefore, the two bacteria should have the same capacity to grow in both samples. However, this is not verified, this behaviour can be justified by the arrangement of bacteria cell walls, which differ from each other. In the case of *K. pneumoniae*, it has a cell wall composed of lipopolysaccharides, lipids A, a core oligosaccharide and an O-antigen specific polysaccharide sidechain. In contrast to many other core structures of Enterobacteriaceae, the oligosaccharides of *K. pneumoniae* O:1 and O:8 do not have phosphate residues [35]. In turn, *E. coli* presents in its cell lipopolysaccharides O antigens and H antigens [36]. Another difference between the cores of *K. pneumoniae* and *E. coli* resides in the presence of galacturonic acid in the outer core region of the former and the presence of hexose containing in the latter can facilitate the adhesion to textile substrate with different wettability [35].

In the case of the fungus *C. albicans*, *Care Us* shows antifungal activity after 168 h of application, the values of antimicrobial activity are also higher than 2 log, however, they are the lowest in table 2. This fact is explained by the lower growth in the control sample, as reported for *K. pneumoniae*.

In general, *Care Us* has the same capacity to inhibit the growth of gram-positive, gram-negative bacteria and fungus, on a porous surface for 168 h of application, since it showed, in the majority tests, where values are well above 2 logs for antimicrobial activity.

3.4.3. Evaluation of antimicrobial activity on non-porous surfaces

For the non-porous samples from aluminium the standard ISO 22196:2007 was used and results are presented in Fig. 10 and Table 3, for samples.

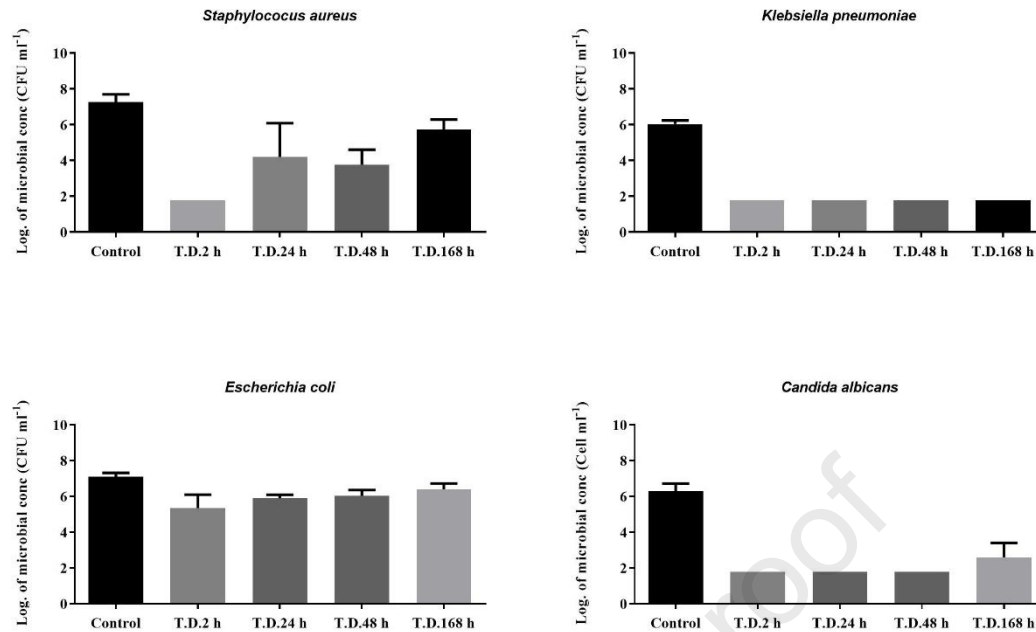


Fig. 10 - Microbial concentration logarithm after 24 h contact between aluminium sample and microorganism, for different time of disinfectant application, samples with no standard deviation indicate that no microbial growth was observed.

Table 3 - Antimicrobial activity for aluminium sample, for *S. aureus*, *K. pneumoniae*, *E. coli* and *C. albicans*, for different time of disinfectant application

		Aluminium Sample			
Time Points (h)		2	24	48	168
Microorganism	<i>S. aureus</i>	> 5	> 3	> 3	< 2
	<i>K. pneumoniae</i>	> 4	> 4	> 3	> 4
	<i>E. coli</i>	< 2	< 2	< 2	< 1
	<i>C. albicans</i>	> 3	> 3	> 3	> 2

In a first analysis of Fig. 10 and Table 3, it is possible to verify that for *S. aureus* Care Us has antimicrobial activity values greater than 2 log up to an application time of 48 h. Focusing on gram-negative bacteria, for *K. pneumoniae*, the results show antimicrobial activity up to 168 h of application, because all values are clearly above 2 log, while for *E. coli* no antimicrobial activity, for none of the time points. A possible explanation is that the disinfectant may not be able to enter/fix itself on the bulk structure

as they are non-porous surfaces and more resistant strains, as in the case of *S. aureus* and *E. coli*, the antimicrobial effect is reduced. This result corroborates the agar diffusion disk test performed by *Care Us*, which shows smaller zones of inhibition for these strains. Moreover, in the case of *E. coli* the lack of antimicrobial activity can also be explained by the development of high-density colonies. The fungi analysis shows in the case of *C. albicans* that *Care Us* has antifungal activity up to 168 h of application.

Additionally, the adhesion phenomena of microorganisms are influenced by material properties and ambient conditions. In the adhesion process, the hydrophobicity, roughness and chemical composition of the surfaces are factors to consider [37].

3.4.4. Evaluation of antimicrobial activity on everyday surfaces

After conducting the tests on porous and non-porous surfaces, under aseptic conditions, it was decided to carry out tests on everyday surfaces, since this would be the closest way to the real use of *Care Us*, the disinfectant under study. Therefore, the ISO 18593:2004 standard was used, and the results are presented in Fig. 11.

Fig. 11 shows a large number of colonies of bacteria and fungi, in the control sample, which corresponds to a surface where *Care Us* was not applied. In the other samples where the disinfectant was applied with the respective times, there was almost total decrease in the number of bacteria and a total decrease in fungi.

The surfaces analysed for the test were plastic surfaces. These are considered non-porous surfaces, so the results obtained in this test agree with the results obtained in tests carried out under aseptic conditions on non-porous surfaces.

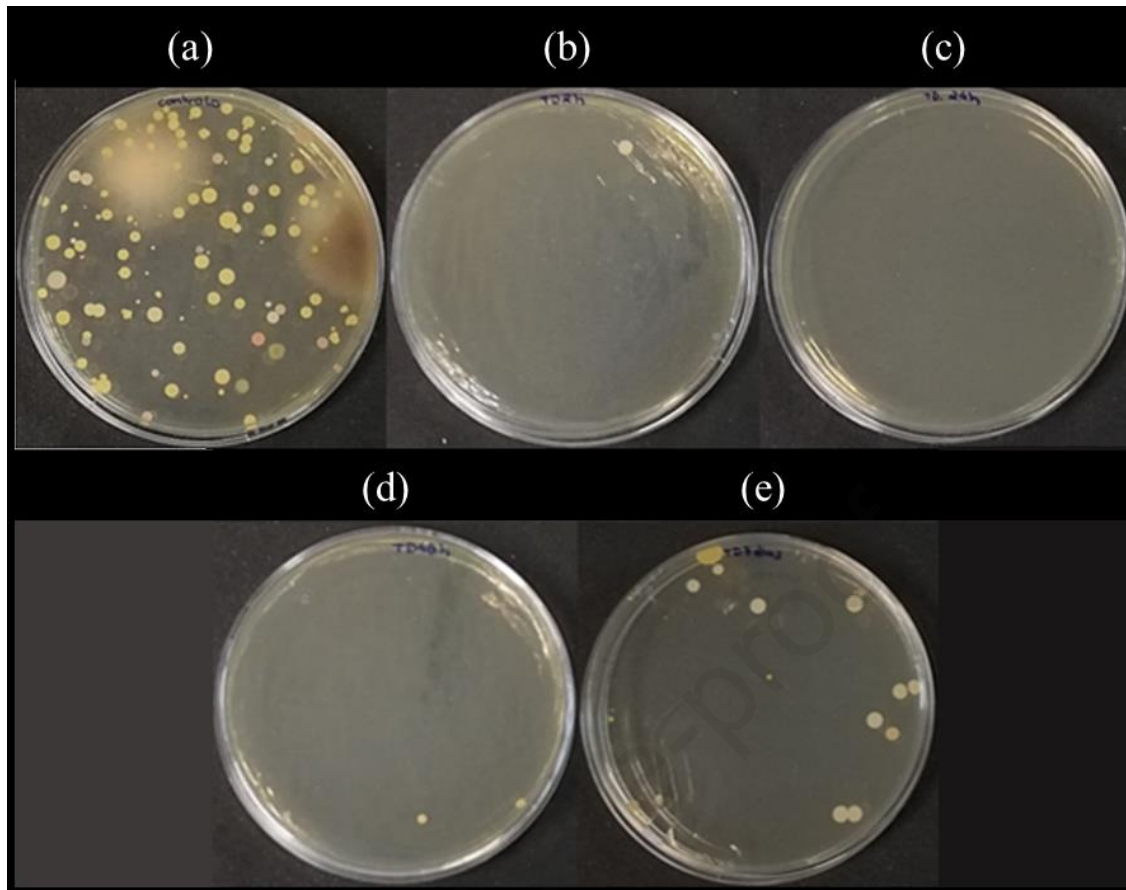


Fig. 11- Results of test on everyday surfaces, a) control sample, b), c), d) and e) disinfection time of 2 h, 24 h, 48 h and 168 h, respectively.

In Fig. 11, it is noteworthy the high inhibition of microbial growth in the samples referring to application times of 2, 24 and 48 h. As for the 168 h samples, it is possible to visualize a reduction of bacterial colonies and a total fungal inhibition, being therefore possible to affirm that *Care Us* presents the same performance both under aseptic conditions and under real conditions.

4. Conclusion

The present work aimed to study the effectiveness and durability of a long-lasting multi-surface disinfectant.

In the accelerated stability test, it was concluded that there was a loss of about 80% of the active substance after 14 days. Despite the high percentage of active substance loss, *Care Us* maintains antimicrobial activity after 14 days of testing, corresponding to 2 years of storage. In antimicrobial activity tests, *Care Us* demonstrated antimicrobial activity for disinfectant application times of up to 7 days, for all microorganisms under study, on porous surfaces. On non-porous surfaces, the antimicrobial activity of the disinfectant against *K. pneumoniae* and *C. albicans* was maintained up to 7 days of application. However, against *S. aureus*, the samples showed antimicrobial activity up to 2 days after application of the disinfectant. Regarding *E. coli*, it proved to be more resistant and in this sense the samples did not show antimicrobial activity for any times of the application. The last point of study focuses on the antimicrobial activity on everyday surfaces where results of total fungal inhibition and high bacterial inhibition on surfaces were obtained after 7 days of disinfectant application. Thus, this study demonstrated that *Care Us* has a potential antimicrobial action of at least 7 days, as for its storage, more studies will be necessary to reduce the losses of active substances.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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HIGHLIGHTS

Care Us is the under study disinfectant developed by the company *Success Gadget*

The disinfecting active substances are hydrogen peroxide and ethanol.

Care Us has an antimicrobial activity for a long period of application.

Care Us maintains antimicrobial activity for up to 2 years of storage.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: