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Direct and Residual Microbicidal Efficacy of Various Antiseptics against Multi-Drug Resistant Bacteria

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Abstract

Background: Infections in ICU's patients are known to often originate from the colonization of wounds by the patient's endogenous microbiota, and to eventually lead to secondary sepsis. Aim: to compare *in vitro* the direct and residual effects after different exposure times of 4% chlorhexidine, and of 0.1% and 0.04% polyhexanide (in gel and solution forms), on ATCC-microorganisms, and too, on bacterial strains obtained from ICU patients. Methods: We used wild multi-drug resistant strains recently obtained from the wounds of patients hospitalized at ICU and reference strains from the American Type Culture Collection (ATCC). Chlorhexidine 4% was studied as a reference solution. The direct and residual effects of the 0.1% and 0.04% polyhexanide, in gel and solution forms, were analyzed using cotton germ carriers. To evaluate the direct effect, we exposed the strains to the antiseptic. To assess the residual effect, the germ-carriers were impregnated with antiseptic and were allowed to dry before we contaminated them. We inoculated the germ carriers in a culture medium with an inhibitor of antiseptic effect to count the number of surviving microorganisms. Findings: 0.1% Polyhexanide solution proved a direct and residual efficacy after 24 hours equivalent to 4% chlorhexidine. Is very important to highlight that this great efficacy did not change according to whether they were ATCC or multidrug-resistant strains. Conclusions: 0.1% polyhexanide demonstrated a great direct and residual efficacy (like 4% chlorhexidine), against multi-drug resistant strains isolated from ICU's patients. Moreover, due to its few cytotoxicity against keratinocytes and fibroblasts can be an optimal antiseptic for burns, wounds or ulcers.

Keywords

Antimicrobial Efficacy, Antiseptic, Multi-Drug Resistant Bacteria, Tissue

Toxicity, Wounds

1. Introduction

In recent years, we have witnessed numerous advances in the management of ICU patients that have resulted in reduced mortality rates. However, they have also brought about other effects, such as extended hospital stays and nosocomial infections, the latter being one of the most important causes of morbidity and mortality among critical patients [1] [2].

Infections in critical patients are known to often originate from the colonization of wounds or ulcers by the patient's endogenous microbiota, and to eventually lead to secondary sepsis. However, this microbiota experiences changes throughout the patients' hospital stay dependent on its duration and the antibiotic pressure, but also on contamination from other sources, both environmental and by microbiota of the health personnel who perform several dressing procedures in critical patients [3] [4]. Infections in these patients are more frequently related to the following microorganisms: Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii, and Klebsiella pneumoniae. Nevertheless, these pathogens often develop mechanisms of resistance to antibiotics, which makes controlling their infections difficult. Particularly the last two bacteria are significantly associated with increased mortality rates [5] [6] [7]. Some authors have calculated the prevalence of the different germs in ICU patients, finding that Klebsiella sp. accounts for 20% of infections in these patients, which is similar to that of Acinetobacter sp. and Pseudomonas sp., but well above that of Staphylococcus sp. However, multi-drug resistance is most frequently associated with Acinetobacter sp., followed by Staphylococcus sp. and Klebsiella sp. [8]. The increase in microbial resistance in recent years has led to the development of new strategies for the treatment of infected wounds as precursors to sepsis in ICU patients. To date, the search for optimal treatments for these wounds and for avoiding antibiotic selective pressure is one of the most researched matters in the care of ICU patients. Wound cleansing is a routine procedure in the care of critical patients, involving the use of numerous products that can be classified according to their method of use, duration, direct effect, residual effect, etc. [9]. These products, such as water-based solutions, isotonic saline solutions, Ringer's lactate solution, or a wash solution containing a surfactant facilitate the removal of dead tissue, dirt, microorganisms, and biofilms. Nevertheless, the addition of an antiseptic agent to the irrigation fluids enhances their decontamination effect, thus decreasing the need for antibiotic treatments and, therefore, their side effects. Unlike antibiotics, which act against a broad spectrum of bacteria, antiseptic agents destroy bacteria, fungi, and, in some cases, even viruses and prions. In addition to their antimicrobial spectrum not being reduced to bacteria, these products must also meet other criteria, such as a lack of bacterial resistance se-

lection, an immediate and residual effect, a lack of interference with cutaneous products, and, finally, a lack of cytotoxic effects on the patient's healthy tissue [10]. Most antiseptics have an implicit cytotoxic effect, not only on bacteria but also on the epithelium to be preserved and whose growth we intend to promote, as this is of great relevance in the treatment of wound patients [11]. Of all antiseptics, 0.5% chlorhexidine digluconate and polyhexanide solutions have proven to be well tolerated by patients. Chlorhexidine digluconate is the most used antiseptic solution; however, it has been associated with the highest cytotoxicity in both in vitro studies performed on fibroblast cultures and chorioallantoic membrane tests [12]. In a contrary sense, Polyhexanide solutions, at a concentration of 0.1% and 0.04%, have shown scarce cytotoxicity in chorioallantoic membrane tests. Understandably, we are interested in determining whether their antimicrobial efficacy is similar.

Study objective: we designed an experimental, *in vitro*, study to compare the direct and residual effects after different exposure times of 4% chlorhexidine (reference antiseptic) and of 0.1% or 0.04% polyhexanide (in both gel and solution forms), on ATCC bacteria (reference microorganisms) and multiresistant-bacteria obtained from wounds of ICU patients.

2. Material and Methods

1) Products under evaluation: 5

- Lavanid gel (0.04% polyhexanide), Serag-Wiessner, Naila (Germany).
- Lavanid 2 solution (0.04% polyhexanide), Serag-Wiessner, Naila (Germany).
- Prontosan gel (0.1% polyhexanide), B Braun Medical AG, Sempach (Switzerland).
- Prontosan solution (0.1% polyhexanide), B Braun Medical AG, Sempach (Switzerland).
- Hibiscrub skin cleanser (4% chlorhexidine), Regent Medical, Lancashire (England).

2) Microorganisms: 9

- ATCC strains: *E. coli* 25,922, S. epidermidis 12,228, S. aureus 25,195, and P. aeruginosa 10,145.
- Multi-drug resistant microorganisms, obtained from ICU's patients: *E. coli*, MR-S. aureus, P. aeruginosa, K. pneumoniae, and Acinetobacter nosocomialis.

3) Methods: The direct and residual effects of the antiseptic products on the microorganisms cited above were analyzed after different exposure times using cotton germ-carriers, given that they yield similar results to corpse skin germ-carriers, which, as we proved in a previous study, are correlated with the efficacy results obtained for antiseptic agents used *in vivo* [13]. Inhibition of the antiseptic effect was achieved with the same inhibitor used in previous studies: nutrient broth (Difco) with 6% Tween-80, 0.5% sodium bisulphite, and 0.5% sodium thiosulfate [14]. The inhibition of all products under evaluation

was assayed under the specified test conditions, achieving a correct inhibition of their antimicrobial efficacy. For this assessment, we used the 4 ATCC strains referred to earlier.

a) Direct effect: A germ carrier was contaminated with one of the cited microorganisms and allowed to dry for 30 minutes. The antiseptic product under evaluation was then added to the carrier (if the antiseptic was a solution, each germ-carrier was impregnated with 100 microliters of the product, whereas if it was a gel, the germ carrier was dipped into a drop of the gel in such a way as to ensure that the entire carrier was covered by the product). The third step consisted of inhibiting the antiseptic after the exposure times described below and inoculating the carriers in a culture medium to count the number of surviving microorganisms after each contact time. The inoculation could be performed directly into nutrient broth containing the inhibitor, or in a 1:100 dilution in all cases except for the controls, in which case it was diluted at a ratio of 1:100 and 1:10,000. The number of surviving microorganisms obtained after each exposure time was divided by that obtained in the control carrier (germ-carrier soaked in still water instead of an antiseptic), and, finally, the log10 reduction was calculated. Studies requiring a 24-hour exposure had to include a second control carrier in addition to the ones described earlier. Both the germ carrier under study requiring a 24-hour exposure and the control carrier were stored in an empty and sterile Petri dish closed with a lid to prevent them from desiccating to the extent possible. Given that desiccation destroys microorganisms present in both the germ carrier under study and its respective control carrier, the log reduction was then calculated after 24 hours with respect to the results of the second control. The experiments were performed in duplicate, and the log reduction was calculated based on the weighted means (diluted and undiluted) obtained for each of the Petri dishes incubated for the specified exposure times (at least 6 results per product and microorganism). This method was repeated for all products and microorganisms under study, exposing the microorganisms to the antiseptic for 15 min, 2 h, or 24 h.

b) Residual effect: As in the previous method, the germ carriers were impregnated with antiseptic; however, in this case, they were allowed to dry for 2 h or 24 h at room temperature. Following these exposure times, we contaminated the germ carriers soaked with what remained of the residual antiseptic. We then allowed each of the antiseptic products to act for 2 h, inhibited them, and inoculated each carrier to count the number of surviving microorganisms after each exposure time. The rest of the procedure was the same as that described for the direct effect method, although the antiseptics were only left to act for a single exposure time of 2 h.

c) Statistical method: With the log reductions obtained for the different products and microorganisms under study, we calculated their centralization values (mean and standard deviation [SD], median, and 25th and 75th percentiles) and performed an analysis of variance (ANOVA) applying the Bonferroni correction

to determine whether or not there were differences between the different products, doses, exposure times, types of microorganisms, etc. analyzed in terms of their direct and indirect effects.

3. Results

The mean log10 reduction in the number of colony-forming-units (CFU) observed in the inocula performed for the analyses of the direct and residual effects was 5.95 ± 1.03 (15 min and 2 h) and 4.66 ± 0.7 (24 h) in the direct effect analysis, and 5.47± 0.63 (2 h) and 5.06 ± 0.62 (24 h) in the residual effect analysis.

Tables 1-4 and Figure 1 summarize the direct and residual effects of the 5 antiseptics used for the specified exposure times. In these, total elimination of the inoculum was indicated as a log10 reduction = 6. The following may be highlighted about their direct effects in the post-hoc comparison: After 15 min of exposure, 0.04% polyhexanide and 0.1% polyhexanide did not achieve the same log10 reduction as 4% chlorhexidine; however, 0.1% polyhexanide proved to be more effective than 0.04% polyhexanide. No differences were observed between

Table 1. Direct effect: mean (and standard deviation) of logarithmic reduction of 9 microorganisms by 5 antiseptics after 15 min.

Microorganisms	Products				
	Lav-gel	Lav-sol	Pront-gel	Pront-sol	Hib
E. coli (2)	1.28 (0.48)	0.44 (0.23)	2.51 (1.28)	1.76 (1.18)	5.25 (0.94)
A. nosocomialis	2.84 (0.21)	3.05 (0.04)	2.88 (0.19)	2.68 (0.01)	6 (0)
K. pneumoniae	1.23 (0.09)	1.15 (0.36)	1.89 (0.17)	1.68 (0.06)	3.84 (0.44)
S. aureus (2)	1.25 (0.46)	0.91 (0.5)	1.58 (0.51)	1.43 (0.49)	2.99 (0.31)
P. aeruginosa (2)	1.18 (0.43)	0.78 (0.6)	2.13 (0.42)	3.23 (1.18)	5.09 (1.04)
S. epidermidis	3.14 (0.21)	1.91 (0.01)	5.3 (0.98)	2.66 (0.08)	6 (0)
Total Gram+	1.88 (1.04)	1.24 (0.65)	2.82 −2	1.84 (0.74)	3.99 (1.57)
Total Gram−	1.5 (0.71)	1.11 −1	2.34 (0.78)	2.39 (1.13)	5.1 −1

Lav gel: 0.04% polyhexanide gel; Lav-sol: 0.04% polyhexanide solution; Pront-gel: 0.1% polyhexanide gel; Pront-sol: 0.1% polyhexanide solution; Hib: 4% chlorhexidine solution. Total destruction of the control inoculum was indicated as a log10 reduction = 6. Post hoc comparison (in order of efficiency): 1st 4% chlorhexidine solution; 2nd 0.1% polyhexanide (no differences gel-solution); 3rd 0.04% polyhexanide (no differences gel-solution). The efficacy rank of these antiseptics did not change according to whether they were ATCC strains or strains obtained from the wounds of patients.

Table 2. Direct effect: mean (and standard deviation) of logarithmic reduction of 9 microorganisms by 5 antiseptics after 2 hours.

Microorganisms	Products				
	Lav-gel	Lav-sol	Pront-gel	Pront-sol	Hib
E. coli (2)	2.86 (1.13)	2.34 (1.37)	6 (0)	3.27 (0.21)	6 (0)
A. nosocomialis	3.34 (0.35)	5.22 (1.06)	6 (0)	6 (0)	6 (0)
K. pneumoniae	2.23 (0.06)	6 (0)	2.05 (0.03)	2.89 (0.27)	6 (0)
S. aureus (2)	2.45 (1.44)	1.32 (0.3)	4.19 (2.11)	3.64 (2.72)	6 (0)
P. aeruginosa (2)	2.11 1.02)	1.67 (0.78)	4.55 (1.66)	5.61 (0.79)	6 (0)
S. epidermidis	3.21 (0.05)	3.11 (0.06)	5.35 (0.91)	5.01 (1.4)	6 (0)
Total Gram+	2.71 (1.18)	1.91 (0.95)	4.57 (1.78)	4.1 (2.3)	6 (0)
Total Gram−	2.58 (0.93)	3.21 (2.02)	4.86 (1.71)	4.44 (1.4)	6 (0)

Lav gel: 0.04% polyhexanide gel; Lav-sol: 0.04% polyhexanide solution; Pront-gel: 0.1% polyhexanide gel; Pront-sol: 0.1% polyhexanide solution; Hib: 4% chlorhexidine solution. Total destruction of the control inoculum was indicated as a log10 reduction = 6. Post hoc comparison (in order of efficiency): 1st 4% chlorhexidine and 0.1% polyhexanide gel (no differences); 2nd 0.1% polyhexanide solution (no differences gel-solution); 3rd 0.04% polyhexanide (no differences gel-solution). The efficacy rank of these antiseptics did not change according to whether they were ATCC strains or strains obtained from the wounds of patients.

the gel and solution forms of these products.

- After 2 h of exposure, the results of 0.1% polyhexanide in gel form did not differ significantly from those of 4% chlorhexidine, while the results of the other 3 products were worse. 0.1% polyhexanide continued to be better than 0.04% polyhexanide after this exposure time, and no differences were observed when comparing both formulations for each product.
- After 24 hours of exposure, only 0.04% polyhexanide solution was seen to be significantly worse than 4% chlorhexidine, as the effects of the other 3 antiseptic products were similar to those of this reference antiseptic. The posthoc comparison of the residual effects revealed the following after 2 and 24 hours of exposure:
- The results of 0.1% polyhexanide solution were like those of 4% chlorhexidine (the rest of the products yielded worse results).
- The solution forms of both products were better than the gel forms *in vitro*.
- The efficacy of 0.1% polyhexanide gel was like that of 0.04% polyhexanide solution, and 0.04% polyhexanide gel was the worst of the 5 antiseptic products analyzed in terms of efficacy.

Table 3. Direct effect: mean (and standard deviation) of logarithmic reduction of 9 microorganisms min by 5 antiseptics after 24 hours.

Microorganisms	Products				
	Lav-gel	Lav-sol	Pront-gel	Pront-sol	Hib
E. coli (2)	6 (0)	3.79 (1.49)	6 (0)	6 (0)	6 (0)
A. nosocomialis	6 (0)	6 (0)	6 (0)	6 (0)	6 (0)
K. pneumoniae	6 (0)	6 (0)	6 (0)	6 (0)	6 (0)
S. aureus (2)	4.77 (0.46)	2.63 (0.87)	6 (0)	6 (0)	6 (0)
P. aeruginosa (2)	3.03 (1.73)	2.77 (1.73)	6 (0)	6 (0)	6 (0)
S. epidermidis	6 (0)	6 (0)	6 (0)	6 (0)	6 (0)
Total Gram+	4.84 (0.37)	3.42 (1.39)	6 (0)	4.39 (0.94)	6 (0)
Total Gram−	4.34 (1.32)	3.85 (1.52)	6 (0)	6 (0)	6 (0)

Lav gel: 0.04% polyhexanide gel; Lav-sol: 0.04% polyhexanide solution; Pront-gel: 0.1% polyhexanide gel; Pront-sol: 0.1% polyhexanide solution; Hib: 4% chlorhexidine solution. Total destruction of the control inoculum was indicated as a log10 reduction = 6. Post hoc comparison (in order of efficiency): 1st 4% chlorhexidine and 0.1% polyhexanide (no differences gel-solution); 2nd 0.04% polyhexanide (no differences gel-solution). The efficacy rank of these antiseptics did not change according to whether they were ATCC strains or strains obtained from the wounds of patients.

- We also assessed whether the type of microorganism affected the efficacy of the 5 antiseptic products studied (results from ANOVA analysis):

1) When comparing ATCC strains with clinical strains, the latter tended to be more sensitive (log10 = 0.1 - 0.6) both in terms of the products' direct and residual effects, although this difference was not statistically significant.

2) Gram-negative bacteria were significantly more susceptible than Gram-positive bacteria (log10 = 1.1 - 2), but only in terms of the products' residual effects, as no significant differences were observed between the direct effects caused by both types of microorganisms.

4. Discussion

The onset of infections or septicemia during the patients' clinical stay has been found to be significantly associated with increased mortality rates. In 2009, Herruzo *et al.* [3] proved that septicemia and pneumonia multiplied this risk by 3.8 and 2.84, respectively. This association demonstrated the importance of controlling

Table 4. Residual effect: mean (and standard deviation) of logarithmic reduction of 9 microorganisms by 5 antiseptics after 2 hours.

Microorganisms	Products				
	Lav-gel	Lav-sol	Pront-gel	Pront-sol	Hib
E. coli (2)	0.65 (0.43)	4.66 (1.54)	4.1 (0.55)	5.36 (0.73)	5.67 (0.65)
A. nosocomialis	1.38 (0.07)	3.22 (1.02)	6 (0)	6 (0)	6 (0)
K. pneumoniae	1.14 (0.22)	1.48 (0.02)	2.65 (0.1)	4.05 (0.92)	6 (0)
S. aureus (2)	0.17 (0.11)	0.8 (0.16)	0.8 (0.27)	3.03 (0.37)	4.64 (1.56)
P. aeruginosa (2)	1.25 (1.33)	2.98 (2.22)	4.44 (1.92)	6 (0)	6 (0)
S. epidermidis	0.34 (0.21)	0.83 (0.1)	1 (0.44)	3.67 (0.74)	6 (0)
Total Gram+	0.22 (0.15)	0.81 (0.14)	0.86 (0.31)	3.24 (0.55)	5.1 (1.4)
Total Gram−	1.05 (0.79)	3.33 (1.8)	4.29 (1.47)	5.46 (0.86)	5.89 (0.37)

Lav gel: 0.04% polyhexanide gel; Lav-sol: 0.04% polyhexanide solution; Pront-gel: 0.1% polyhexanide gel; Pront-sol: 0.1% polyhexanide solution; Hib: 4% chlorhexidine solution. Total destruction of the control inoculum was indicated as a log10 reduction = 6. Post hoc comparison (in order of efficiency): 1st 4% chlorhexidine and 0.1% polyhexanide solution (no differences); 2nd 0.1% polyhexanide gel and 0.04% polyhexanide (no differences gel-solution). The efficacy rank of these antiseptics did not change according to whether they were ATCC strains or strains obtained from the wounds of patients.

wound colonization to avoid the risk of septicemia originating from a skin wound [1]. Antiseptics play a fundamental role in the treatment of wounds, ulcers and burns, provided they are effective antimicrobial and non-cytotoxic.

Several studies have also shown that the use of chlorhexidine gels in patient hygiene regimens contributes to reducing the incidence of secondary bacteremia and sepsis in critical patients [15]. In his study [16], Popp proved that the implementation of specific protocols to reduce catheter-related bacteremia, urinary tract infections, and ventilator-associated pneumonia in burn patients improved their preventive capacity when accompanied by a daily hygiene regimen including the application of chlorhexidine gluconate solutions.

The current clinical guidelines indicate the use of antiseptics to prevent the colonization (including multiresistant strains) of acute wounds, decolonize these skin breaks, treat infected wounds, and prepare chronic wounds for debridement [17]. Thus, it is imperative that large quantities of these antiseptic products be available at a local level to inhibit the growth of microorganisms, prevent the

Table 5. Residual effect: mean (and standard deviation) of logarithmic reduction of 9 microorganisms by 5 antiseptics after 24 hours.

Microorganisms	Products				
	Lav-gel	Lav-sol	Pront-gel	Pront-sol	Hib
E. coli (2)	0 (0)	1.99 (0.11)	2.28 (0.62)	3.85 (1.33)	4.6 (0.79)
A. nosocomialis	1.32 (0.03)	2.12 (0.01)	6 (0)	6 (0)	6 (0)
K. pneumoniae	0.94 (0.08)	0.83 (0.08)	2.11 (0.03)	4.08 (1.29)	6 (0)
S. aureus (2)	0.16 (0.32)	0.93 (0.21)	0.31 (0.08)	2.34 (0.94)	3.66 (1.57)
P. aeruginosa (2)	0.84 (1.01)	1.84 (0.82)	3 (2.31)	3.72 (1.47)	4.05 (1.13)
S. epidermidis	0.05 (0.07)	1.05 (0.07)	0.79 (0.33)	3.28 (0.16)	6 (0)
Total Gram+	0.12 (0.26)	0.97 (0.18)	0.47 (0.29)	2.65 (0.88)	4.11 (1.4)
Total Gram−	0.65 (0.74)	1.77 (0.62)	2.94 (1.61)	4.04 (1.2)	4.55 (0.83)

Lav gel: 0.04% polyhexanide gel; Lav-sol: 0.04% polyhexanide solution; Pront-gel: 0.1% polyhexanide gel; Pront-sol: 0.1% polyhexanide solution; Hib: 4% chlorhexidine solution. Total destruction of the control inoculum was indicated as a log10 reduction = 6. Post hoc comparison (in order of efficiency): 1st 4% chlorhexidine and 0.1% polyhexanide solution (no differences); 2nd 0.1% polyhexanide gel and 0.04% polyhexanide solution (no differences); 3rd 0.04% polyhexanide gel. The efficacy rank of these antiseptics did not change according to whether they were ATCC strains or strains obtained from the wounds of patients.

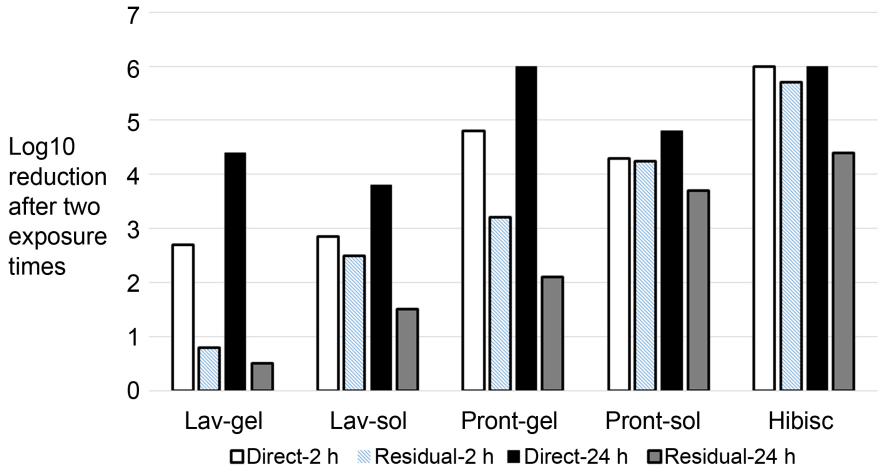

Figure 1. Comparison of direct and residual effects of 5 antiseptics against 9 microorganisms on germ carriers.

systemic effects caused by high doses of antibiotics, and reduce the incidence of microbial resistance to these drugs. However, antiseptics exhibit intrinsic cytotoxic activity against the wound itself and the cells involved in its healing.

Some current antiseptics are known to have a broad antibacterial spectrum and several studies have studied, moreover, its effects on keratinocytes and fibroblasts [18] [19] [20] [21]. For example, Hirsch *et al*. [18] demonstrated that polyhexanide has a good antibacterial effect with lower cytotoxic properties against keratinocytes and fibroblasts when analyzed in vitro. Hence, it is especially interesting to determine whether this lower cytotoxic effect of polyhexanide is of relevance to confirm that the drug could be an effective antiseptic against bacteria that are frequently involved in infections of patients treated at ICUs. The cited study specifically analyzed the antiseptic efficacy of the drug against Enterococcus faecalis, Escherichia coli, Staphylococcus aureus, and Pseudomonas sp., but only using ATCC strains. Given that it is widely known that ATCC strains do not exhibit the same clinical behavior as wild strains of the same species recently isolated from clinical patients [22] [23] [24], this effect may not be extrapolated to a real clinical setting. In addition, these earlier studies [18]-[24] did not evaluate the direct effect of the antiseptic as a cleansing agent in comparison with its residual effect on the wound as a protective agent against future contamination. Because of this, for our study, we considered analyzing both ATCC strains and their corresponding wild multiresistant strains obtained from cultures of the wounds of real patients treated at our ICUs, and evaluating both, the direct and the residual effect, of the antiseptics on the wounds.

The results of our analysis proved that 0.1% polyhexanide solution is a good antiseptic for burns, wounds or ulcers and that its direct and residual efficacy after 24 hours of exposure is equivalent to that of 4% chlorhexidine, as it destroys practically all microorganisms present in the area on which it is applied. Because of its direct effect, 0.1% polyhexanide gel can also be used to treat these wounds, as the efficacy of both pharmaceutical forms of 0.1% polyhexanide was seen to be similar, although the residual effect of the gel form was worse than that of the solution. This is probably because the gel did not impregnate the entire surface of the germ carriers as well as the solutions, thus being less effective following their contamination a few hours after the gel's application for the residual effect assay.

In the direct effect assay, although the gel did not soak the germ carriers as well as the solutions, the amount of product absorbed by the carriers was enough to destroy most of the microorganisms present in them. Furthermore, although the ATCC strains were expected to be more sensitive to the products under study than their corresponding wild strains, this difference was not seen to be statistically significant (they might have been statistically significant if we had increased the number of strains). In addition, although we did observe differences between the residual effects caused by Gram-positive bacteria versus Gram-negative ones, we did not find any between the direct effects caused by

these microorganisms, probably also due to our sample size (N).

Finally, we have demonstrated a great increase in the healing rate of chronic wounds when using "double therapy", liquid plus gel, [25] by improving the residual effect of the liquid product with the gel. Therefore, an attempt can be made to demonstrate whether the same occurs with the 0.1% polyhexanide formulation, first cleaning the injured skin with the liquid solution and then applying the gel, which is left to act until the next cure so that the wound epithelializes with the lowest possible microbial load.

5. Conclusions

- 0.1% Polyhexanide has a lower bactericidal speed than 4% chlorhexidine, but if it is left to act for long periods of time (as required in burns or chronic wounds), the direct and residual efficacy of both is similar against multi-drug resistant bacteria isolated from ICU-patients. Moreover, due to its few cytotoxicity against keratinocytes and fibroblasts can be an optimal antiseptic for burns, wounds or ulcers.
- Of all the antiseptic products tested, 0.04% polyhexanide (solution or gel) proved to have a worse antiseptic direct and residual efficacy, and been less useful.
- 0.1% polyhexanide, solution and gel, due to its great direct and residual efficacy, could be used as a "double therapy" in the treatment of wounds, burns or ulcers.

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Contributors

The individual contribution of each author:

José Ramón Martínez-Méndez: conception and design of the study, acquisition of data, analysis and interpretation of data. Revising the article critically for important intellectual content and final approval of the version to be submitted.

Rafael Herruzo: conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article and final approval of the version to be submitted.

Angela Ojeda: analysis and interpretation of data, drafting the article and final approval of the version to be submitted.

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Conflicts of Interest

There is no potential conflict of interest regarding the publication of this manu-

script.

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