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Topical formulations based on polyhexamethylene hydrochloride guanidine for surgical field antisepsis

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Abstract: As surgical site infection can prolong the patient's hospital stay, increase morbidity and mortality rates and medical and hospital expenses, justifying the search for new effective and low-cost antiseptic drugs is justifiable. Thus, the objective of the present study was to analyze, by direct clinical and microbiological examination, the topical antiseptic effect of a solution based on polyhexamethylene hydrochloride guanidine (PHMGH) at 0.5% on the intact skin surface of sheep and cats, aiming at its use in operative field (previous and definitive antisepsis), compared to 0.5% alcoholic chlorhexidine and association with this. The topical solutions did not cause any skin damage, regardless of species. The PHMGH demonstrated an antiseptic effect equivalent to chlorhexidine, however, the association between PHMGH and alcoholic chlorhexidine was more effective compared to isolated products. It is concluded that the topical products tested were harmless to the skin surface; PHMGH can be a preventive and less expensive option in skin antisepsis and that the association between PHMGH and alcoholic chlorhexidine was more effective in microbiological control when compared to the isolated products tested, however, more research will be essential to investigate the potentiation of these, as well as the performance of PHMGH in the presence of body fluids.

Key words: Antimicrobial activity, surgery, infection, skin, synthetic polymer

1. Introduction

Infections in the surgical field can prolong hospital stay and increase patient morbidity and mortality, in addition to increasing medical and hospital expenses [1–3]. In this context, regardless of the species, the main source of infection is the direct inoculation of the patient's own skin microbiota at the surgical site; thus, surgical interventions must be necessarily initiated with the use of skin antiseptic products in the incision area and adjacent regions, with minimal damage and irritation, aiming at considerable reduction of transient and resident microorganisms [4]. Chlorhexidine digluconate is the most commonly used product for this purpose, and at high concentrations it exerts a bactericidal function and, at low concentrations, it acts as a bacteriostatic [5].

Despite advances in the pharmaceutical industry, each medicament presents distinct therapeutic response and side effect, none being fully effective. In addition, it is considered that the common and indiscriminate use of antiseptics in the health area can cause resistance of microorganisms [4]. Thus, studies that propose the investigation of new broad-spectrum drugs are justified in the surgical area [6], aiming at promising and less costly preventions; in this context, the synthetic polymer polyhexamethylene hydrochloride guanidine (PHMGH) [7,8] stands out, both for isolated use and in association with established commercial products.

PHMGH is a cationic synthetic polymer from the guanidine family, whose synthesis occurs by polycondensation of hexamethylenediamine chloride with dicyandiamide [9, 10]. Cationic polymers such as guanidine-based polymers are of great interest and widely used due to their high antibacterial and antiviral activity and low toxicity to humans [11]. PHMGH is becoming increasingly popular due to its broad range of antimicrobial activity. This polymeric guanidine presents significant antibacterial [12–14] and antifungal activities [15] in vitro experimental models, and even at low concentrations, demonstrates rapid and prolonged performance [10,16,17], and no developed resistance has been reported [10].

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Guanidine polymer inhibits bacterial growth by attacking them through electrostatic attraction between cationic guanidio groups and anionic groups on the cell surface of bacteria [18]. After attaching to bacteria cells, guanidine polymer induces bacterial membrane collapsed and intracellular components leaked thereafter [13]. PHMGH diffuses through the cellular membrane and binds to the cytoplasmic membrane forming a complex with the phospholipid molecules of the lipid bilayer, destabilizes the osmotic equilibrium and destructs cytoplasmic membrane, causing leakage of cell. It strongly reacts with nucleic acid, in both cases creatingionic bindings with monophosphate groups present in the bacterial cell and in the nucleic acid [19].

Given the high number of elective and emergency procedures and surgical complications, together with the scarcity of scientific data on the in vivo activity of polyhexamethylene guanidine hydrochloride, the objective of this study was to analyze the topical and antiseptic effect of a solution based on PHMGH at 0.5%, on intact skin surface of sheep and cats, mimicking previous antisepsis in operative sites. Also, this study aims to compare it with commercial alcoholic chlorhexidine at 0.5% and associate it with this same drug, aiming to analyze possible potentiation of the products.

2. Material and methods

2.1. Obtention of the polymer and topical solutions preparation

PHMGH (Akwaton) was provided by Fosfaton Akwaton International Ltd. (Canada).

Two topical solutions were formulated, in one of them, the polymer was diluted in deionized water to reach the final concentration of 0.5% and in the other, added at this same concentration to commercial alcoholic chlorhexidine at 0.5% (Riohex - Indústria Farmacêutica Rioquímica Ltda., São José do Rio Preto - SP).

The solutions were kept in a closed container at room temperature.

2.2. Ethical aspects

The study protocol was approved by the Ethics Committee on Animal Care of the University of Franca - UNIFRAN (Approval no. 9968110518). The choice of animal species was based on the different surgical environments normally adopted in the operative routine of each one, in addition to microorganisms in the surgical field. Furthermore, the different body regions most accessed surgically in each animal category were considered.

2.3. Experimental design

2.3.1. Sheep

Twenty-four Santa Inês sheep were used, male and female, whole and castrated, of varying age and weight, from the Veterinary Hospital of UNIFRAN and kept in collective pens, with water, commercial feed and hay ad libitum. The inclusion criterion of animals in the research was the absence of skin lesions diagnosed by direct clinical inspection.

The experiment was carried out in the stalls, mimicking the outdoor surgical environment, according most procedures in this species, with the team involved wearing a cap, mask, surgery pajamas and sterile gloves. Without the use of anesthesia, the sheep were mechanically restrained for extensive shaving of the left lateral region, close to the flank, with the aid of a professional clipper.

2.3.2. Cats

Twenty-four male cats, intact, of varying age, breed and weight, free of skin lesions, belonging to client tutors of the UNIFRAN Veterinary Hospital, were used.

The experiment was conducted in the operating room with the team involved wearing attire (cap, mask, shoe cover, surgery pajamas, surgical gown and sterile gloves). With the use of dissociative general anesthesia, trichotomy of the middle third of the abdomen of all cats was performed with a professional clipper.

Then, in all sheep and cats, a trichotomized area of 8 cm^2 was delimited, proceeding with the basal microbiological collections (M0) with a sterile swab in Stuart transport medium (Olen - Model K41-0102, China).

Subsequently, antisepsis was performed on the demarcated and intact areas, with sterile gauze soaked in the tested topical formulations, recommending three series/three times in each antimere, recommending from the center to the edges. For this, both sheep and cats were randomly distributed into four groups of six animals: GPHMGH - group submitted to skin antisepsis with a 0.5% polyhexamethylene guanidine hydrochloride-based solution; GCL - antisepsis with 0.5% commercial alcoholic chlorhexidine; GPHMGH-CL - group submitted to skin antisepsis with 0.5% polyhexamethylene hydrochloride guanidine solution associated with 0.5% commercial alcoholic chlorhexidine and GSF - untreated control group: antisepsis with 0.9% saline solution (Brasmédica S.A. Pharmaceutical Industry Ltd, São Paulo, Brazil).

After 1 min (M1), 5 min (M5), 10 min (M10), 40 min (M40) and 60 min (M60) of skin antisepsis with the different products, microbiological samples were collected, following the same criteria as M0.

2.4. Clinical analysis

The animals were inspected by direct clinical examination for the presence or absence of dermal changes (hyperemia, erythematous plaque, pruritus, among other manifestations that expressed discomfort and/or adverse reactions to dermal applications), resulting from the action of the topical products tested. Clinical results were expressed descriptively.

2.5. Microbiological analysis

The microbiological samples from all analyzed experimental moments were individually identified, sent and processed, by blind study, at the Research Laboratory in Applied Microbiology (LAPEMA) of UNIFRAN, following conventional quantitative and qualitative techniques [20].

2.5.1. Dilution of microbiological samples

For sample dilution, the swabs were immersed in test tubes containing 3 mL of 0.9% saline solution (Brasmédica S.A. Pharmaceutical Industry Ltd) for six consecutive hours and incubated in aerobic conditions at 36.5 ºC overnight; after that, an aliquot of 100 µL was transferred from each tube to a tube containing 0.9 mL of saline solution, carrying out an initial dilution of 10 times. In total, three serial dilutions were performed on the scales of 10^{-1}, 10^{-2} and 10^{-3}, from the original undiluted sample (10^{0}).

From each dilution previously obtained, 100 µL of this saline solution was placed in Petri dishes containing the culture media (Merck KGaA, Darmstadt, Germany) Brain and Heart Infusion (BHI) and MC Conkey (for aerobic bacteria), blood agar (for microaerophiles) and Saboraud Agar (for fungi and yeasts). The inoculated aliquots on the plates were seeded by the spreading technique, with the aid of a Drigalski loop and, after, for the growth of aerobic bacteria, the culture media remained 48 h in an aerobic oven, while for the microaerophilic they remained in microaerophilic oven with 5% CO_2 for 48 h. For fungal growth, the plates were kept for seven days in an aerobic oven, all incubated at 37 ºC.

2.5.2. Count of colony forming units per milliliter (CFU/mL)

At the end of the incubation, a quantitative analysis of the microorganisms was performed by counting the colony forming units per milliliter (CFU/mL). For this, initially, the colony growth homogeneity was evaluated in the three dilutions obtained (10^{-1}, 10^{-2} and 10^{-3}).

At the lowest dilution scales, the colony forming units were counted by estimation, requiring the division of the Petri dish into four parts, where only one was counted and multiplied by the remaining parts. At the end, the number of CFU/mL was converted, according to the dilution used: CFU/mL = number of colonies counted/sample dilution factor × inoculated volume.

The fungal colonies were observed fresh and added a drop of potassium hydroxide (Sigma-Aldrich St. Louis, USA) in a small amount of colony. In addition, a drop of cotton blue reagent (Sigma-Aldrich) was added to another small amount of colony.

Quantitative assays were performed in triplicate and results expressed in CFU/mL.

2.5.3. Colony isolation

After counting the CFU/mL, the colonies were isolated and purified. For this, the Petri dishes with the greatest morphological diversity were chosen and the visual identification of the different morphotypes was performed according to the colony characteristics, such as color, border shape, size and texture of the colony.

Isolation was performed with the aid of a sterile platinum loop, where a portion of the chosen morphotype was transferred and seeded through the depletion technique, in Brain and Heart Infusion culture medium (BHI, Merck KGaA). The plates were identified according to the description of the chosen morphotype, and incubated under the same conditions as the processes previously carried out. After the incubation time of the plates, the growth and presence of morphotypes different from the desired one was verified, and thus, the colony purification process was carried out, where a well-isolated portion of the colony was transferred from the isolation plate and drained again in a plate containing Brain and Heart Infusion medium (BHI, Merck KGaA), until a completely pure colony was obtained.

These isolated colonies were stored in test tubes containing Brain and Heart Infusion broth (BHI, Merck KGaA) and stored aerobically at 36.5 °C for use in the following stages of the study.

2.5.4. Identification of microorganisms

After the colony purification process, a qualitative analysis of microorganisms was performed. For this, the isolates were transferred to Petri dishes containing MacConkey agar selective medium (Kasvi, São José dos Pinhais, Brazil) to favor the growth of gram-negative microorganisms. The plates were incubated in an inverted position at 36.5 °C for 24 h, after which the reading was performed to assess bacterial growth.

Qualitative analysis was performed semiautomatically, using the BBL Crystal Enteric/Nonfermenter and BBL Crystal Gram-Positive Kits (BD Life Sciences, East Rutherford, NJ, USA) for gram-negative and gram-positive bacteria, respectively.

For the identification procedure, pure isolates seeded on Trypticase Soy Agar (Kasvi) with 5% sheep blood (Laborclin, Pinhais, Paraná) were used. Thus, a well-isolated colony (from 2 to 3 mm in diameter) of each morphotype to be identified was selected with the aid of a sterilized swab. Then, the selected colonies were transferred to tubes containing the fluid for the preparation of the bacterial suspension of each Kit, and the turbidity was performed, corresponding to 0.5 on the McFarland scale.

Then, the inoculated panels were placed downwards in an incubator without CO_2, with 40% to 60% humidity, for 20 h for gram-negative bacteria and 24 h for gram-positive bacteria. Reading was performed using the BBL Crystal

Light Box (BD Life Sciences), following the specifications of each Kit. The profile number of the isolates, obtained by reading the BBL Crystal Light Box, as well as the results of the necessary complements, was arranged in the BD BBL Crystal MIND software to obtain the final identification.

Fungal colonies were analyzed under an optical microscope (Leica Microsystems DMLB, Wetzlar, Germany) in a 400x objective. Qualitative tests were performed in triplicate and the results expressed in a descriptive way.

2.6. Statistical analysis

Quantitative microbiological results from different times and experimental groups were statistically compared using the analysis of variance test (ANOVA) for repeated measures, adopting a significance level of 5% ($p \leq 0.05$), using the Graphpad Prism 8.0 software.

3. Results

3.1. Sheep

3.1.1. Clinical

No sheep showed skin symptoms secondary to the application of topical products.

3.1.2. Microbiological

At different times of analysis, the qualitative microbiological assay detected the presence of gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Corynebacterium pseudotuberculosis*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Staphylococcus epidermidis*) and yeasts (*Rhodotorula sp*).

Regarding the quantitative microbiological assay, there was a statistically significant reduction in the count of colony forming units at each analysis time ($p < 0.001$) and, during these periods, the tested products showed considerable effectiveness, except for the saline solution. In this context, in 50% of the GPHMGH sheep, the product started to act after the M10 of the topical application, while in the GCL sheep, only in the M40 and M60. On the other hand, in all of the GPHMGH-CL, the combination of topical products was 100% effective since M10. Furthermore, the association between polymer and commercial antiseptic was more promising in the control of skin microorganisms, compared to isolated products (Figure 1).

Up to M5, GPHMGH did not show a statistically significant difference compared to GSF ($p = 0.13$), however, in the subsequent microbiological analysis moments, this difference was significant, indicating the antiseptic effect of the synthetic polymer, even at low concentration (Table 1).

According to Table 1, at different times of analysis, there was no significant statistical difference between GPHMGH compared to GCL ($p = 1.000$), demonstrating similar microbiological control of the polymer and the commercial antiseptic. On the other hand, there was a significant microbiological reduction of GPHMGH-CL compared to GSF ($p < 0.003$), as well as GCL when compared to GSF ($p < 0.016$).

3.2. Cats

3.2.1. Clinical

Similar to sheep, at different times, cats from all groups did not show any cutaneous symptoms.

3.2.2. Microbiological

The qualitative microbiological assay, at different experimental times, revealed gram-positive

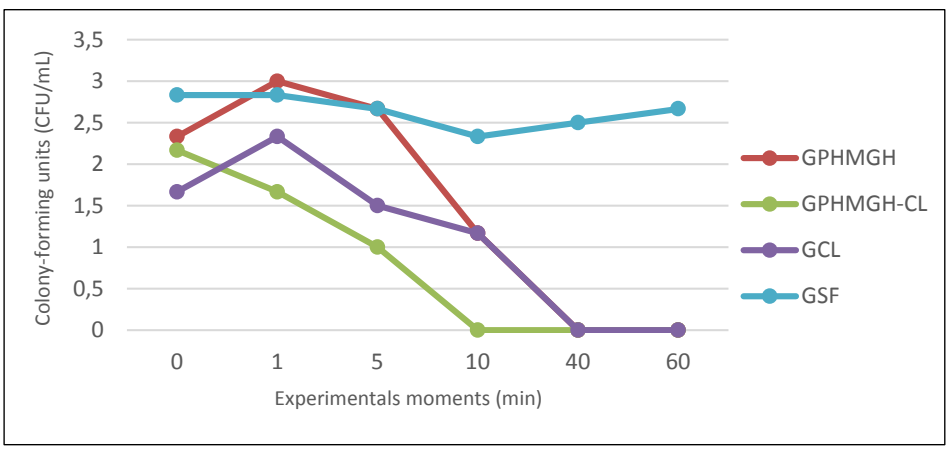

Figure 1. Graphic representation of colony forming units/mL in intact skin of 24 Santa Inês sheep, at baseline (0) and after 1, 5, 10, 40 and 60 min of skin antisepsis with different topical solutions. **GPHMGH**: group submitted to skin antisepsis with a 0.5% polyhexamethylene guanidine hydrochloride-based solution (n = 6); **GPHMGH-CL**: skin antisepsis with the association of 0.5% polyhexamethylene guanidine hydrochloride and 0.5% commercial alcoholic chlorhexidine (n = 6); **GCL**: skin antisepsis with 0.5% commercial alcoholic chlorhexidine solution (n = 6) and **GSF**: skin antisepsis with 0.9% commercial saline solution (n = 6).

Table 1. Means and standard deviations (M ± SD) of colony forming units (mL) in intact skin of 24 Santa Inês sheep, at baseline (M0) and after 1 (M1), 5 (M5), 10 (M10), 40 (M40) and 60 (M60) min of skin antisepsis with different topical solutions.

Moments (min)	GROUPS (n=6)	M ± SD
M0	GPHMGH	2.333 ± 1.0
	GPHMGH-CL	2.167 ± 0.4
	GCL	1.667 ± 0.8
	GSF	2.833 ± 0.9
M1	GPHMGH	3.000 ± 1.6
	GPHMGH-CL	1.667 ± 0.8[a]
	GCL	2.333 ± 0.5
	GSF	2.833 ± 0.9
M5	GPHMGH	2.667 ± 1.7
	GPHMGH-CL	1.000 ± 0.8[a]
	GCL	1.500 ± 0.8[a]
	GSF	2.667 ± 1.2
M10	GPHMGH	1.167 ± 1.6[a]
	GPHMGH-CL	0 ± 0[a]
	GCL	1.167 ± 0.4[a]
	GSF	2.333 ± 1.2
M40	GPHMGH	0 ± 0[a]
	GPHMGH-CL	0 ± 0[a]
	GCL	0 ± 0[a]
	GSF	2.500 ± 1.3
M60	GPHMGH	0 ± 0[a]
	GPHMGH-CL	0 ± 0[a]
	GCL	0 ± 0[a]
	GSF	2.667 ± 1.2

GPHMGH: group submitted to skin antisepsis with a 0.5% polyhexamethylene guanidine hydrochloride-based solution (n = 6); **GPHMGH-CL**: skin antisepsis with the association of 0.5% polyhexamethylene hydrochloride guanidine and 0.5% commercial alcoholic chlorhexidine (n = 6); **GCL**: skin antisepsis with 0.5% commercial alcoholic chlorhexidine solution (n = 6) and **GSF**: skin antisepsis with 0.9% commercial saline solution (n = 6).

[a] Significantly different from the GSF at each time point of analysis ($p \leq 0.05$).

(*Corynebacterium diphtheriae*, *Staphylococcus saprophyticus*, *Bacillus mentagrophytes*, *Staphylococcus aureus* and *Gemella haemolysans*) and Gram-negative (*Enterobacter sakazakii*) bacteria.

Similar to sheep, there was a statistical reduction in the count of colony forming units at each analysis time ($p < 0.001$) and the tested products were effective throughout these periods, with the exception of saline solution.

In five cats (83.3%) of GPHMGH, the polymer began to act on M10, identical to those of GCL. The combination of products (GPHMGH-CL) significantly reduced microorganisms by 66.7% in M1, being 100% effective in M5 (Figure 2).

Similar to sheep, GPHMGH showed no statistical difference compared to GSF up to M5, however, in the following moments, the microbiological reduction was discrepant, confirming the antiseptic characteristic of the synthetic polymer.

Still equivalent to the results of sheep, in the feline species there was no statistical difference of GPHMGH in contrast to GCL at different times, demonstrating microbiological similarity of both products. There was a considerable statistical microbiological reduction between GPHMGH-CL compared to GSF ($p < 0.001$), as well as between GCL and GSF ($p < 0.001$). Furthermore, the association between polymer and commercial antiseptic was more promising in the control of skin microorganisms, compared to isolated products (Table 2).

4. Discussion

Due to scarcity of scientific studies evaluating the antiseptic effect of PHMGH in dermatology, combined with its low cost and already demonstrated properties in vitro [2,12,16,18], the current research aimed to evaluate, by clinical and microbiological examination, the isolated action of a topical solution containing a commercial version of this compound at 0.5% (PHMGH - Akwaton) and associate with commercial chlorhexidine at the same concentration.

In view of the innumerable microorganisms involved in skin microbiota, in vivo tests using different species animals are indispensable in the investigation of new dermatological products [21]. Thus, the choice of experimental models was based on the high incidence of elective and emergency surgical procedures in small, medium and large animals, as well as postoperative complications resulting from infections at the surgical site [6]. Different resident skin microorganisms were also considered, given the difference in maintenance and handling of each animal species.

For shaving, a professional clipping machine was chosen toavoid skin microlesions that could occur with the use of a shaving blade, which could predispose the resident microbiota to multiply, as reported by Silva et al. [22]. In this sense, the established body regions aimed to simulate the places commonly accessed surgically in the selected species.

The choice of PHMGH in aqueous solution was based on its high solubility in water, as well as to enable comparison with the standard antiseptic of the market; similar to concentration [2,10,12]. In this context, Privitera et al. [1] reported that aqueous topical solutions require more time to dry on the skin, however, in the study

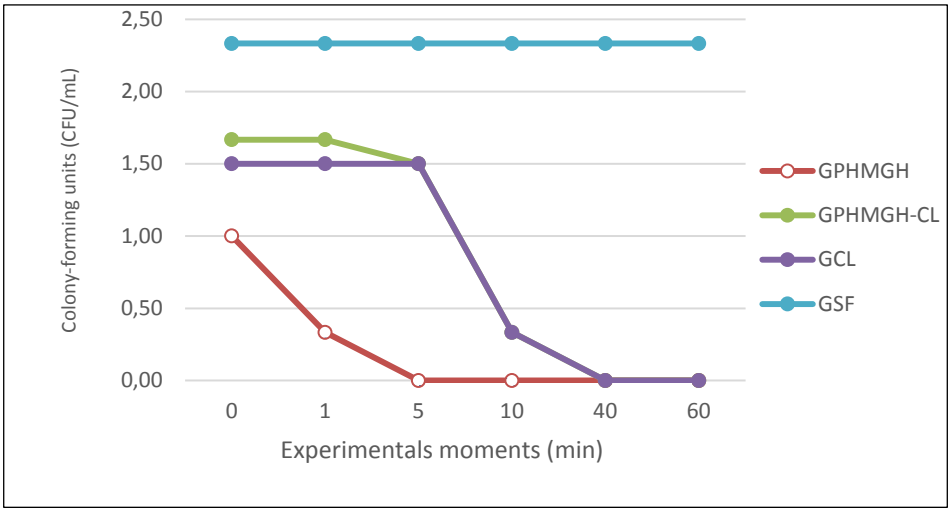

Figure 2. Graphic representation of colony forming units/mL in intact skin of 24 cats, at baseline (0) and after 1, 5, 10, 40 and 60 min of skin antisepsis with different topical solutions. **GPHMGH**: group submitted to skin antisepsis with a 0.5% polyhexamethylene guanidine hydrochloride based solution (n = 6); **GPHMGH-CL**: skin antisepsis with the association of 0.5% polyhexamethylene guanidine hydrochloride and 0.5% commercial alcoholic chlorhexidine (n = 6); **GCL**: skin antisepsis with 0.5% commercial alcoholic chlorhexidine solution (n = 6) and **GSF**: skin antisepsis with 0.9% commercial saline solution (n = 6).

conducted here, such a time demand was not observed in the GPHMGH animals that could interfere with the preparation of the site to be later surgically manipulated.

When testing the antiseptic effect of 0.5% PHMGH on experimentally induced superficial skin wounds in rats, Dias et al. [23] observed that no animal treated showed clinical toxicity symptoms, indicating that the polymer can be harmless to the skin´s surface in this concentration, corroborating the clinical results of this study. Although alcoholic chlorhexidine has low irritability [4,24], Zhang et al. [2] reported cutaneous clinical signs in humans such as erythematous plaque, pruritus, and hyperemia; on the other hand, in the current research, at the different moments evaluated, the sheep and cats belonging to the GPHMGH and GPHMGH-CL were asymptomatic, indicating that the products based on the synthetic polymer did not cause irritation and other local damage, being innocuous to the skin surface. In addition, PHMGH, because it is devoid of volatile organic compounds, is odorless [12,14], a particularity that can minimize allergic risks and local licks. Given the promising results found in the in the present study, this product alone or associated with alcoholic chlorhexidine can be used prior to the insertion of intravenous catheters and injections of drugs, both in animals and in humans, and not restricted to surgical antisepsis.

According to the scientific literature [25,26], the normal flora of cutaneous microorganisms of domestic animals is diverse, depending on management, food and habitat, including gram-positive, gram-negative bacteria and fungi, thus justifying the realization of previous complementary exams to identify each one of them for further microbiological tests using products that could possibly be used in the future as antiseptics. In accordance with the scientific literature, chlorhexidine showed gradual efficacy against gram-positives [27], gram-negatives and fungi [27], as well as PHMGH [2] and their association, even at low concentrations [12,16,18] and against resistant microorganisms, especially *Staphylococcus aureus*, *Enterococcus faecalis* and *Gemella haemolysans*. They also acted positively against the resistant and gram-negative bacterium *Enterobacter sakazakii*, which has a double outer membrane and, consequently, is more rigid, which acts as a permeability barrier against some antiseptic products [28], reducing its entry into the cytoplasm [12].

When investigating the 0.5% polymer in superficial skin wounds experimentally induced in rats, Dias et al. [23] observed that the product demonstrated the ability to eliminate up to 100% of microorganisms until the fourth day of treatment, whereas, in the intact skin of sheep and cats, the complete reduction in colony-forming unit counts occurred after minutes of the performance of PHMGH, especially with its association with alcoholic chlorhexidine. This polymer´s performance in short periods has already been described by Koffi-Nevry et al. [29], Mathurin et al. [7] and Oulé et al. [14], as well as its prolonged residual effect [17], similar to that evidenced until the last analyzed time of this study, probably due to the substantivity power, remaining linked in its active form to the cutaneous stratum corneum [1,30].

Casually, a slight increase in the number of cutaneous microorganisms was observed in some groups of sheep

Table 2. Means and standard deviations (M ± SD) of colony forming units (mL) in intact skin from 24 cats, at baseline (M0) and after 1 (M1), 5 (M5), 10 (M10), 40 (M40) and 60 (M60) min of skin antisepsis with different topical solutions.

Moments (min)	GROUPS (n = 6)	M ± SD
M0	GPHMGH	1.500 ± 0.5
	GPHMGH-CL	1.000 ± 0
	GCL	1.667 ± 0.8
	GSF	2.333 ± 0.5
M1	GPHMGH	1.500 ± 0.5
	GPHMGH-CL	0.333 ± 0.5[a]
	GCL	1.667 ± 0.8[a]
	GSF	2.333 ± 0.5
M5	GPHMGH	1.500 ± 0.5
	GPHMGH-CL	0 ±0[a]
	GCL	1.500 ± 0.5[a]
	GSF	2.333 ± 0.5
M10	GPHMGH	0.333 ± 0.8[a]
	GPHMGH-CL	0 ± 0[a]
	GCL	0.333 ±0.8[a]
	GSF	2.333 ± 0.5
M40	GPHMGH	0 ± 0[a]
	GPHMGH-CL	0 ± 0[a]
	GCL	0 ± 0[a]
	GSF	2.333 ± 0.5
M60	GPHMGH	0 ± 0[a]
	GPHMGH-CL	0 ± 0[a]
	GCL	0 ± 0[a]
	GSF	2.333 ± 0.5

during the stipulated moments; this fact can be attributed to the environmental conditions of experimental execution (in the field), which favored microbial multiplication [31], despite the fact that the team involved was correctly outfitted. However, regardless of the animal species and experimental location, PHMGH, when isolated, showed progressive efficacy similar to alcoholic chlorhexidine established in the human and veterinary market, and the association between these products potentiated the microbiological effects. Thus, such data propose that the synthetic polymer, alone or in association, may be a promising and less expensive option [12] in the control of cutaneous microorganisms. In addition, there are no reports of PHMGH antimicrobial resistance [32] and, recently, some researchers from the same team [23] described the absence of delayed healing, hepatotoxicity, nephrotoxicity and toxicity after 21 days of using the polymer in superficial skin wounds, experimentally induced in rats.

The beneficial antimicrobial effects of PHMGH found in the present work can be attributed to its mechanism of action on the cell membrane [33,34] in promoting inhibition of enzymes essential for microorganisms growth, in addition to phospholipid degradation [17,35], with coagulation of the cytosol and leakage of its cytoplasmic content, with consequent cell death [10,12,18], which were possibly potentiated when combined with alcoholic chlorhexidine. Still in relation to the mechanism of action of PHMGH, there is also evidence that once inside the cell, the product binds to DNA and other nucleic acids, damaging or inactivating the bacterial genetic material [36]. In addition, Choi, Kim and Lee [16] reported that the polymer acts on fungal plasma membranes inducing the formation of pores, which cause loss of K^+ ions followed by contraction and cell death.

Also in relation to the effect of the polymer, Zhou et al. [9] and Luo et al. [37] described that the PHMGH's antimicrobial action due to the presence of the flexible linear alkyl chain in the polymer that improves the partition capacity in the hydrophobic regions of the phospholipid membranes, damaging the phospholipid bilayer of the microorganisms.

In view of the results obtained in this work, future research is essential to investigate the interaction and physical-chemical stability [5] of the polymer with alcoholic chlorhexidine and the probable potentiation of chlorhexidine and/or of ethyl alcohol due to the addition of PHMGH. It is still prudent to evaluate the antimicrobial activity alone or in association with these products in the presence of body fluids present during surgical procedures.

5. Conclusion

Based on the recommended methodology and the results obtained, it is assumed that, clinically, topical solutions based on polyhexamethylene hydrochloride guanidine were innocuous on the intact skin surface of animals. As for the antiseptic effect, regardless of the experimental environment, the isolated polymer, even at low concentration, showed similarity to commercial alcoholic chlorhexidine, established in human and animal medicine. Also, the microbiological effects obtained with the association of the synthetic polymer with alcoholic chlorhexidine were more promising compared to the isolated products, justifying the need for future research to investigate such potentiation.

The clinical and microbiological results further perspectives in the development of novel products for surgical field antisepsis, using PHMGH as an active ingredient.

Conflict of interest

The authors declare that there are no conflicts of interest.

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