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Effect of Common Antiseptics on Skin Flora in Sheep

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Abstract: The purpose of skin preparation prior to surgery is to rid the surgical site of organic material, hair and oil and to reduce the number of transient and resident microorganisms, thus minimizing the risk of post surgical infections. The purpose of the present study was to evaluate surgical preparation of ovine skin. Four antiseptics were used: 1- Povidine Iodine 1% (PI), 2- Chlorhexidine Gluconate1% (CH), 3- Cetrimide - C 1% (CC), 4- Ethyl Alcohol 70%, Povidine Iodine 1%, Ethyl Alcohol 70% (ABA), 5- Double washing with soap and water (C_1), 6- No additional washing and treatment (C_2). Right and left flanks of five healthy sheep were divided into six sections (10×8 cm) by sticker tapes after initial washing by water and soap and each section was randomly prepared by one of the above mentioned preparations. Samples for bacteriologic count were taken before scrub, immediately after scrub and one hour postscrub and CFUs were evaluated and compared in different groups. A two way repeated measures ANOVA was used for statistical analysis of the data. CFUs were significantly ($p<0.05$) reduced in all groups except C_2 at the postscrub sampling. The highest reduction was in PI and CH groups. One hour after scrub just PI and C_2 did not show any significant difference compared initial sampling ($p<0.05$). It seems that CH is the best antiseptic for preparation of the ovine skin in reducing the number of bacteria.

Key words: Antiseptics, sheep, skin, chlorhexidine, povidine iodine, cetrimide

INTRODUCTION

The increase in post surgical infections, cost and time related to its treatment make the surgery team to be aware of a good aseptic situation before, during and after the surgery. 44000-98000 human death have been reported from surgical infections in USA that made 17-29 million dollars expenses annually (Eggimann and Pittet, 2002).

Routinely, preoperative skin preparation is performed following induction of anesthesia and involves clipping, surgical scrubbing and application of antiseptic. Even with this multistage skin preparation, up to 20% of the skin resident bacteria are still inaccessible to any type of skin disinfectant (Sebben, 1983).

Food animal surgery is generally performed under less than optimal conditions and surgical wounds are frequently contaminated to some degree. Antimicrobial treatment must be used judiciously in food animals because of imposed withdrawal times for milk and slaughter and because of the potential for adverse effects such as allergy, toxicosis, injection site abscesses and the induction of antibiotic-resistant bacteria.

Prevention of infection in surgical wounds is a challenge to all surgeries because true surgical asepsis can never be achieved. Many efforts have been directed at reducing contamination at the operation site and preventive techniques are now well described and quite effective (Romatowski, 1989).

The bactericidal scrub and antiseptic solution that is most effective in reducing bacterial numbers would be indicated for preparing the area for surgery. A long residual activity would also be indicated to help control bacteria on the skin following surgical procedures. With the potential for considerable surface contamination and from bacteria coming up the skin surface from hair follicles, an antiseptic with good residual activity would be indicated to counteract these bacteria (Coolman *et al*., 1998).

The purpose of the current experimental study was to evaluate and compare the bactericidal effect of several antiseptics used commonly as skin pre-surgical preparations in and ovine model.

MATERIALS AND METHODS

Antiseptics: Four different commercial antiseptics and 5 antiseptic preparations were used as the following:

- Povidine Iodine (Iran Kondor, Gorgan, Iran, 78-30-D.T.).

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- Cetrimide-C (Damloran, Borujerd, Iran, 73-15-D.T.).
- Chlorhexidine Gluconate (Fort doge, USA).
- Ethyl Alcohol (Iran Ararat, Tehran, Iran, 4069).

Povidine Iodine, Cetrimide C and Chlorhexidine were used as 1% solution. All dilutions were prepared by adding sterile distilled water to the original solution. Ethyl alcohol was applied as 70% solution.

Animals: Five mixed-breed sheep from both sexes and same age were selected. All animals were dewormed (Albendazole, 7.5* mg kg^{-1}) and housed in controlled situation a week before experiment. Both flanks and chest wall of the animals were clipped and shaved and each side was divided into 3 equal areas (10×8 cm) using paper sticker tape.

Microbiological method: For maximal bacterial recovery from the specimens, swabs were moistened with diluent to obtain the specimens. After obtaining sample with each swab, the sampling tip was completely dissolved in the diluent (1% sodium citrate, 0.05% Tween 80 and 0.07% Lecithin) to deliver the total bacterial population. Ten fold serial dilutions, using phosphate-buffered saline solution with Tween 80, pH 7.4, were prepared, using the sample dissolved in the citrate buffer as the 10^{-0} dilution. Tween 80 and Lecithin were used to neutralize effects of antiseptics. The dilutions were carried out up to 10^{-4}. One milliliter of each dilution was cultured in duplicate on Tripticase Soy Agar (TSA), using the spread plate method (Desrochers *et al*., 1996). The plates were incubated for 48 h at 37°C.

Plates were examined for total colony count. The total number of colony-forming units was determined from a countable plate, which was defined as having between 30 and 300 colonies.

Experimental design: Prepared areas were washed with water and soap and then divided into 3 equal parts in each side by a bandage sticker tape. The surface for each treatment was (10×8 cm) and divided from A1 to A6 in according to the treatment.

Antiseptics in aseptic situation were applied to one of the above mentioned areas. Samplings were done at various times; T_1: After washing with soap, T_2: Immediately after the treatment and T_3: One hour after application of the antiseptic.

Six groups of treatment were

- One minute scrub with Povidin Iodine 1% (PI).
- One minute scrub with Chlorhexidine 1% (CH).
- One minute scrub with Cetrimide - C 1% (CC).
- One minute scrub with Ethyl Alcohol 70%, a minute scrub with Betadine 1% and one minute Scrub with Ethyl Alcohol 70% (ABA).
- One min washing with soap and water (C_1, Positive Control).
- No additional washing and treatment (C_2, Negative control).

Statistical Analysis: A two way repeated measures ANOVA was used to determine whether the two main effects, time and treatment, were significant for the variable Colony Forming Unit (CFU). All pair wise Multiple Comparison Procedures (Student-Newman-Keuls Method) has been used to isolate significant difference in different groups.

A p-value of 0.05 or less was considered significant. Results are presented as mean±standard deviation. Analyses were carried out using the Sigmastat software (Sigmastat, version 1.0, Jandel Corporation).

RESULTS

In combination comparisons, colony counts obtained from specimens collected at the prescrub stages (T_1) were not different. After the scrubbing procedure (T_2), results related to CC, PI, CH, ABA and C_1 groups were different from the C_2. The lowest count results was recorded in CH group that was significantly lower from CC, ABA and C groups but no significant difference were recorded between CH and PI. Also at that stage results obtained after the PI was significantly lower from CC and control groups, but there was not any difference with CH and ABA groups.

At the T_3 sampling time the results from the C_1 group were significantly higher from all other groups, although counts in CC, CH and PI were lower from other groups but no significant difference were recorded among different antimicrobials and C_2 group (p<0.05) (Table 1).

Table 1: Mean of colony counts for each scrub/antiseptic solution combination at the stages of preparation

Groups	T_3 cfu×10^2	T_2 cfu×10^2	T_1 cfu×10^2
(PI)*	24.4±13.57	2.0±0.7¶	36.4±6.1
(CC)*	14.8±6.22¶	11.8±3.9¶	34.6±9.29
(CH)*	17.4±1.67¶	1.0±0.7¶	45.8±4.97
(ABA)*	23.4±4.56¶	4.6±1.67¶	38.8±7.56
(C_1)*	23.4±4.56¶	18.2±1.3¶	40.0±9
(C_2)*	41.6±11.06	41.4±7.99	42.4±11.13

*: Significant changes during time, ¶: Significant difference with T_1 (P<0.05)

DISCUSSION

The purpose of any preoperative scrubbing solution is to rapidly decrease the skin's microflora without damaging the skin. Complete sterilization of the skin is not possible (Desrochers *et al*., 1996). Most of the bacterial infections after surgeries result from bacteria that might be a part of normal skin flora of the animals (Deyoung *et al*., 1990). Considering the vast spectrum of germs that antiseptics can interfere with, it seems prompt usage of antiseptics is more desirable than the use of antibiotics that generally act against special genus or species of the germs (Kalantar-Hormozi and Davami, 2005). All preoperative scrubbing protocols in this study were effective in decreasing the microflora population of the skin in T_2 but different agents had different residual activity in T_3. Based on results of microbial culture, CH and PI were superior to the other groups immediately postscrub, as reported by Culligan *et al*. (2005) and Swaim *et al*. (1991).

When the 4 scrub solutions (PI, CC, CH and ABA) and positive control were evaluated, a downward trend was shown from the prescrub to the post scrub period. Among the all of the above mentioned groups, a downward trend was also seen from T_1 to T_3. Residual counts (T_3) increased in the negative control and the ABA group; whereas CC, PI and CH groups held the residual counts to minimal growth.

One of the most important features of CH is its residual effect, which can last up to 6 h (Garibaldi *et al*., 1988). In dogs, use of alcohol with CH has been reported to decrease this residual effect (Deyoung *et al*., 1990). The percentage of negative cultures after surgery was lower from dogs scrubbed with CH and alcohol compared with CH and saline. However, alcohol may be less likely to become contaminated when used under field conditions or in community containers compared with saline. Iodine preparations like povidine iodine and chlorhexidine have been used for a long time to get benefit from their antibacterial, antifungal and virocidal action as well as their residual activity (Osuna *et al*., 1990).

In three step preparation technique alcohol used as lipid solvent (Sebben, 1983) and povidine iodine used as main antiseptic and finally alcohol again used as iodine washer to reduce possible interaction with wound healing that was previously reported (Iwasawa and Nakamura, 2003). Since negative effects of povidine iodine on wound closure did not confirmed (Goldenheim, 1993) and regarding to results of current study, alcohol washing after povidine iodine scrub make less residual activity, it seems that this step in not necessary in establishing a reasonable antisepsis and residual effect.

Although post scrub and residual activity was reasonable in CC group, but with regard to its inflammatory inducing potential, its negative effect on wound healing may be a limiting factor on its use as a preoperative skin preparation (Guthua *et al*., 2001; Lee, 1997). However, residual activity of CC could be the result of chlorhexidine that is included in Cetrimide-C solution.

CFUs in C_1 group showed that double washing of the skin with soap significantly decreases the number of bacteria on the skin which can be reliable in short term surgeries, but its long term effects on wound contamination has to be evaluated, although effectiveness of surgical area preparation without using antiseptics has been reported previously (Kalantar-Hormozi and Davami, 2005).

Three step antiseptic preparation of alcohol, povidine iodine, alcohol don't have any benefit to povidine iodine alone in decreasing CFUs immediately after scrubbing and one hour after scrubbing. Usage of chlorhexidine is strongly recommended as a routine preparation method. Usage of Cetrimide-C do not have strong antiseptic effect as other iodine preparations in this study and regarding to its skin inflammatory effects is not recommended.

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