COMPARISON OF THE ANTIMICROBIAL EFFICACY OF TOPICAL ANTISEPTIC CREAMS ON CANINE WOUNDS (PRELIMINARY COMMUNICATION)


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Summary


The aim of this study was to evaluate the antimicrobial efficacy of five broad-spectrum topical antiseptic creams on infected canine wounds and their suitability for clinical application in veterinary practice. Five topical antiseptic creams namely, TCP® (containing chlorine, iodine and phenol), Dettol® (containing phenol compounds, chloroxylenol and triclosan), Savlon® (containing chlorhexidine gluconate), Drapolene® (containing benzalkonium chloride and cetrimide) and Bacidin® (containing chlorhexidine) were evaluated on clean wounds, experimentally infected with beta-hemolytic, coagulase-positive Staphylococcus aureus (field isolate) at concentration 10^9 bacterial organisms/mL (1 mL instilled in each wound). Wounds treated with sterile physiological saline solution served as controls.

A total of 72 wounds were evaluated and all treatments were initiated two hours following wound infection and conducted up to day 28. The mean rate of reduction of bacterial colony count (×10^6 bacterial organisms/mL eluted and diluted swab specimen), and the rate of pus discharge were assessed and compared statistically. There were statistically significantly (P<0.05) lower values for the topical antiseptics used in the study compared to controls. Dettol® antiseptic cream that proved to be more effective than others showed a marked mean reduction in bacterial colonies, and by day 21 bacterial counts were reduced to below ×10^6 bacterial organisms/mL (0.0±0.0, P<0.05). The findings suggest that the use of topical antiseptic creams for the initial treatment of full-thickness skin wounds in companion animals was beneficial from a clinical point of view. The sticking of wound dressing which is common to aqueous preparations was not observed in this study, thus making bandage change easier.

Key words: antimicrobial efficacy, topical antiseptic cream, wounds

INTRODUCTION

The use of antiseptics in wound management still remains a controversial issue, with varying advantages and disadvantages. However, antiseptics are agents that destroy or inhibit the growth and development of microorganisms within or on liv-
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ing tissue. Unlike antibiotics that act selectively on a specific target, antiseptics have multiple targets and a broader spectrum of activity that includes bacteria, fungi, viruses, protozoa, and even prions (McDonnell & Russell, 1999; Drosou et al., 2003).

Antiseptics are expected to play an even more important role in controlling microbes in both veterinary and human medical clinical practice (Altemier, 1983; King, 1995). Several antiseptic agents are mainly intended to clean intact skin and are used in the preoperative preparation of patients, prior to intramuscular injections or venous punctures, pre- and postoperative scrubbing in the operating room (Niedner, 1997). When used properly, antiseptics are effective in both the prevention and treatment of wound infections (Dinnen, 1981; Amber & Swain, 1984; Mayer & Tsapogas, 1993; Goldenheim, 1993). Antiseptics are usually the weakest and least toxic among the surface antimicrobials (Kahrs, 1995). Regardless of the use, antiseptics should exert a sustained effect against microorganisms without causing tissue damage (Brown & Zitelli, 1993; Liptak, 1997).

The broad antimicrobial spectrum, absence of bacterial resistance, and few hypersensitivity reactions in patients make antiseptics an attractive alternative to topical antibiotics or antibacterials for wound management (Rosenburg et al., 1976; Gilmore, 1977; Amber et al., 1983; Reybrouck, 1985; Mayer & Tsapogas, 1993; Goldenheim, 1993; Payne et al., 1998).

This study evaluated the antimicrobial efficacy of five broad-spectrum topical antiseptic creams on canine wounds experimentally infected with Staphylococcus aureus, a common field isolate found in the study area, for a 28-day treatment period.

MATERIALS AND METHODS

Animals

The Ahmadu Bello University Institutional Animal Care and Use Committee approved all procedures used in the study. Six Nigerian local dogs, comprising 3 males and 3 females, with an average weight of 12.5 ± 3.5 kg were used in the study. Animals were kept in individual kennels under strict ethical supervision and guidance. Food and water were provided ad libitum.

Preoperative procedures

Dogs were positioned in sternal recumbency, and the hair on the dorsum of the trunk from the cranial thoracic to the coxofemoral region was clipped and prepared for surgery.

On day 0, dogs were medicated with atropine sulfate (at dose rate of 0.05 mg/kg), chlorpromazine (at dose rate of 3.3 mg/kg) and then anesthetized with thiopentone (25 mg/kg). All animals were intubated. Lactated Ringer’s solution was administered i.v. at 11 mL/kg/h during the anesthesia.

Surgery

Six full skin thickness wounds, measuring 6 cm² were created on each side of the trunk 4 cm ventrolaterally to the dorsal midline and 5-cm spaces were allowed apart or between wounds. In each skin defect all tissues including the panniculus muscle were excised. These wounds were designated as subcutis intact wounds (Waldron & Trevor, 2003).
Wound infection

A pure culture of beta-hemolytic coagulase-positive *S. aureus* of dog skin origin (field isolate) at concentration of $10^9$ organisms/mL was used for wound infection. One mL was instilled into each wound and the site was covered with a sterile drape and left for two hours to allow bacteria to invade and infect the tissue before commencement of treatment (Amber et al., 1983; Mertz et al., 1999).

Wound treatment

The cream antiseptic formulations, which include TCP®, Dettol®, Savlon®, Drapolene® and Bacidin® (Table 1), were used for wound treatment. Each cream had 12 wounds to be evaluated upon and a total of 72 wounds were evaluated including the controls. The treatment was randomized at different locations of wounds. Sterile non-adherent gauze pads of about 10 cm² in size had approximately 2.5 mL of the cream antiseptic medication placed aseptically on them, using a sterile 3-mL syringe as applicator. The medicated pads were then placed over the wound. The control wounds were covered with sterile gauze pads soaked in 0.9% physiological saline solution.

Post wound treatment

After surgery and wound treatment, a secondary soft wrap was placed over the primary dressings, such that the bandages

<table>
<thead>
<tr>
<th>Topical antiseptic cream</th>
<th>Composition</th>
<th>Manufacturer</th>
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</thead>
<tbody>
<tr>
<td>TCP®</td>
<td>TCP liquid antiseptic 40% w/w (chlorine 0.4% w/w; iodine B.P. 0.055% w/w; phenol B.P. 0.063%; sodium salicylate B.P. 0.052% w/w with partial elimination of ionisable chlorine); 2,4,4'-trichloro-2'-hydroxydiphenyl ether 0.1% w/w; water miscible creambase 59.9% w/w.</td>
<td>Pfizer Pharmaceuticals</td>
</tr>
<tr>
<td>DETTOL®</td>
<td>Chloroxylenol B. P. 3% w/w; triclosan 0.3% w/w; edetic acid (as potassium salt) 0.2% w/w in a base containing almond 0.1 Ph. Eur. 0.66% w/w and glycerol Ph.Eur. 1.06 % w/w; emulsifying wax, carbomer, terpineol, perfumes 8399, Es9078, 23364, potassium hydroxide solution 50% and water.</td>
<td>Reckitt &amp; Colman Product Ltd.</td>
</tr>
<tr>
<td>SAVLON®</td>
<td>Cetrimide 0.5% w/w and chlorhexidine gluconate 0.1% w/w. Also contains: cetostearyl alcohol, liquid paraffin, methylhydroxy benzoate, propylhydroxy benzoate, perfume and purified water.</td>
<td>Norvartis Consumer Health</td>
</tr>
<tr>
<td>BACIDIN®</td>
<td>Chlorhexidine gluconate B. P. 1% w/w in a non greasy water miscible basis; parabens 0.23% as preservative.</td>
<td>Xepa-soul Patterson (M) Sdn. Bhd.</td>
</tr>
<tr>
<td>DRAPOLENE®</td>
<td>Benzalkonium chloride solution Ph. Eur. 0.02% w/w, cetrimide Ph. Eur. 0.2% w/w. Also contains: white paraffin, cetyl alcohol, polawax, chlorocresol, amaranth, purified water, and lanolin 2% w/w.</td>
<td>Warner Lambert</td>
</tr>
</tbody>
</table>
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extended from the folds of the flanks to the axillary region. A criss-cross suspender-like pattern were made across the pectoral and lower cervical areas of each dog using the secondary wrap. Two-inch adhesive tapes were applied as tertiary bandage wrap, to prevent it from drying and to preserve the moist wound micro environment. Side braces consisting of aluminum splint rods were incorporated into the bandages to prevent molestation by the dogs. Wound dressings were then changed daily for the first three days and thereafter on each other day. The animals were monitored for any sign of pain or systemic involvement throughout the treatment period.

Wound infection assessment
A tentative diagnosis of wound infection was made based on the presence of erythema, swelling and purulent exudates. Wounds were assessed based on the amount of exudates and bacterial colonies counts. These parameters were chosen because of ease of assessment and for the wound micro environment to remain intact. Exudates were graded and scored at each bandage change as none ($+_=-0$), small ($+, =3$), moderate ($++, =6$), high ($+++, =9$), throughout the 28-day treatment period.

Quantitative bacteria culturing of wounds were performed immediately after creating wounds on days 1, 3, 6, 9, 15, 21 and 28. Swab specimens for bacterial cultures were collected before cleaning of wounds by means of sterile sticks, which were rolled over the wound each time and then aseptically placed in a tube containing 5 mL saline (Lee et al., 1988; Mertz et al., 1999). Using a 100-fold dilution, 0.1 mL were finally pipetted and inoculated on nutrient agar plate and grown aerobically at 37°C for 24 hours. Bacterial colonies were counted as $\times10^5$ bacterial organisms/mL (Lee et al., 1988; Mertz et al., 1999).

Statistical analysis
Duncan’s multiple range tests, was used to determine the statistical significance and differences between the means of observed exudates and bacterial colonies counts.

RESULTS
The exudation of experimental wounds was considerable during the first four days and decreased as the wound healing process progressed (Table 2).

Marked exudation was seen in wounds treated with normal saline (which served as control) and continued throughout the treatment period. Its scores by days 21 and 28 were $3.9\pm0.34$ and $3.1\pm0.30$, respectively, the corresponding bacterial counts being $(147.6\pm8.7)\times10^5$/mL and $(98.1\pm6.8)\times10^5$/mL.

Wounds treated with Dettol® showed significantly minimal discharges as compared with other wound treatments and by day 21, there were no discharges from these wounds (score $-0.0\pm0.0$; $P<0.05$ vs controls) (Table 2).

There was a significant reduction in pus discharge and in bacterial colony counts in wounds treated with the various topical antiseptics as compared to controls (Table 3).

Dettol® treated wounds also showed a marked mean reduction in bacterial colonies and by day 21 (Table 3) bacterial counts were reduced to below $\times10^5$ bacterial organisms/mL ($0.0\pm0.0$; $P<0.05$ vs controls).

The values of bacterial colony counts for TCP® ($0.0\pm0.0$), Drapolene® ($0.0\pm0.0$) and Savlon® ($0.0\pm0.0$) creams by day 28 showed reduction up to zero bacterial or-
### Table 2. Pus discharge from experimental wounds, at each bandage change, following treatment (in days, D) with physiological saline solution (PSS, control) or with various topical antiseptic creams (mean values ± standard error). Exudates were graded and scored at each bandage change as none (-, = 0), small (+, = 3), moderate (+ +, = 6), high (+ + +, = 9)

<table>
<thead>
<tr>
<th>Topical antiseptic creams</th>
<th>Days of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D0</td>
</tr>
<tr>
<td>TCP*</td>
<td>0.0±a</td>
</tr>
<tr>
<td>DETTOL®</td>
<td>0.0±a</td>
</tr>
<tr>
<td>DRAPOL®®</td>
<td>0.0±a</td>
</tr>
<tr>
<td>SAVLON®®</td>
<td>0.0±a</td>
</tr>
<tr>
<td>BACIDIN®®</td>
<td>0.0±a</td>
</tr>
<tr>
<td>PSS (control)</td>
<td>0.0±a</td>
</tr>
</tbody>
</table>

* significant difference vs corresponding control values at P<0.05.

### Table 3. Bacterial colony counts ($x 10^2$) from experimental wounds, at each bandage change, following treatment (in days, D) with various topical antiseptic creams (mean values ± standard error).

<table>
<thead>
<tr>
<th>Topical antiseptic creams</th>
<th>Days of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D1</td>
</tr>
<tr>
<td>TCP®</td>
<td>228±4.29±1</td>
</tr>
<tr>
<td>DETTOL®®</td>
<td>141±8±22.6</td>
</tr>
<tr>
<td>DRAPOL®®®</td>
<td>197±5±26.1</td>
</tr>
<tr>
<td>SAVLON®®®</td>
<td>206±3±36.9</td>
</tr>
<tr>
<td>BACIDIN®®</td>
<td>264±7±40.6</td>
</tr>
<tr>
<td>PSS (control)</td>
<td>631±2±54.2</td>
</tr>
</tbody>
</table>

* significant difference vs corresponding control values at P<0.05.
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organisms/mL, being significantly lower compared to controls at P<0.05.

DISCUSSION

The major role of antiseptics in wound management is to prevent infection by inhibiting further microbial invasion of the tissues, thus giving the body a chance to repair itself (Amber & Swain, 1984; Brown & Zitelli, 1993, Liptak, 1997; Drosou et al., 2003).

It has been established that microbial pathogens delay wound healing through several different mechanisms, such as persistent production of inflammatory mediators, metabolic wastes and toxins, and maintenance of the activated state of neutrophils, which produce cytolytic enzymes and free oxygen radicals (Laato et al., 1988; Pope, 1993). In addition, bacteria compete with host cells for nutrients and oxygen necessary for wound healing (Rodeheaver, 1997).

*S. aureus* is the commonest wound pathogen, and of the organisms used to evaluate antiseptics, *S. aureus* is considered to be the least susceptible to antiseptic agents. The ability to kill *S. aureus*, gives the antiseptic agent a broader spectrum of activity (Liptak, 1997; Mertz et al., 1999). However, regardless of their mode of use, antiseptics should exert a sustained effect against microorganisms without causing tissue damage (Brown & Zitelli, 1993; Doughty, 1994; Mertz et al., 1999).

Alcohols, acetic acid, chlorhexidine, chlorine and chloro compounds, iodine and iodine compounds, phenols (including chloroxylenols), hydrogen peroxide and quaternary ammonium compounds have bactericidal activity as antiseptic agents. Our results for these antiseptics in infected wound agrees with previous evaluations (Amber et al., 1983; Swain & Lee, 1987; Lemarie & Hosgood, 1995; Rodeheaver, 1997). However the previous studies employed aqueous antiseptics rather than topical creams preparations.

Considering the number of wounds per animal, there could be the possibility of systemic involvement, however, it was not observed in this study. When we compared the topical antiseptic cream preparations in infected wounds, the Dettol® cream antiseptic had the most powerful bactericidal activity, followed in descending order of activity by Drapolene®, TCP®, Savlon® creams, with Bacidin® cream showing the weakest activity in reducing bacterial load.

Generally, the physical and chemical properties of this antiseptic’s active substances explain their mode of action. Dettol® cream contains phenol compounds, chloroxylenol and triclosan as active ingredients. They have a wide spectrum of activity against bacteria, viruses, fungi, but a minimal sporocidal activity (Jeffery, 1995). They act on the cytoplasmic membranes, producing leakage and disruption of membrane transport (Denyer & Stewart, 1998).

TCP® cream contains chlorine, iodine and phenol at appropriate proportions. Chlorine acts by oxidation of peptide links and denaturation of proteins (Marris, 1995). However, its activity is usually affected by pH and presence of organic matter (Dychdala, 1991). Iodine acts by decreasing the oxygen requirements of aerobic microorganisms (Marris, 1995). It also interacts preferentially with the proteins of the cytoplasmic membrane (Marris, 1995). Though, bacterial resistance to iodine has been reported (Chapman, 1998), pH has little effect on its antimicrobial efficacy,
but blood reduces the efficacy by converting it to a non-bactericidal iodide.

Drapolene® cream contains benzalkonium chloride (a quaternary ammonium compound) and cetrimide. Benzalkonium chloride acts by binding irreversibly to the phospholipids and proteins of the cytoplasmic membrane of microbes, impairing permeability (Marris, 1995; Denyer & Stewart, 1998). It is far more effective against Gram-positive than Gram-negative bacteria, possesses a narrow margin of safety and can fail when exposed to resistant microorganisms (Terleckyj et al., 1995; Jeffery, 1995). Cetrimide—a cationic detergent has been reported to be toxic to fibroblasts at low concentrations (Brown & Zitelli, 1993; Terleckyj et al., 1995). This may account for the slow rate of bacterial activity seen in wounds treated with Savlon® and Drapolene® antiseptic creams.

Savlon® and Bacidin® contain chlorhexidine gluconate at different concentrations. Chlorhexidine acts by interfering with the function of bacterial cellular membranes and its primary site of action are the cytoplasmic membranes (Barrett-Bee et al., 1994; Marris, 1995; Denyer & Stewart, 1998). Chlorhexidine is more effective against Gram-positive than Gram-negative bacteria and exhibits a bacteriostatic effect against some bacteria (Larson, 1995; Jeffery, 1995). The potential for the development of bacterial resistance to chlorhexidine seems low, but it has been reported (Larson, 1995; Chapman, 1998).

The control wounds treated with normal saline showed significantly increased exudation (mean score 3.1±0.30; P<0.05), and presence of bacterial organisms—(98.1±6.8)×10^5 bacterial colonies (P<0.05) up to day 28. The reduction of exudation and bacterial concentration in antiseptic-treated wounds was due to the activity of the topical antiseptic creams on the bacterial organisms.

On the basis of the results of this study, there are clinically beneficial effects in the use of topical antiseptic creams for the initial treatment of full-thickness skin wounds in companion animals. The sticking of wound dressing which is common to aqueous preparations was not observed in this study, which makes bandage change easier. Consequently, this establishes their usefulness in management of naturally acquired wounds. The use of antiseptics could help decreasing the rate of antibiotics usage and the development of resistance by microorganisms and thereby preserving their advantage for clinically critical situations. As seen from this research, the role of antiseptics on wounds and wound care management should be considered and a more detailed research should be carried out on the mode of their delivery and formulations.

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