Research Article

Standardization of Surgical Site Preparation with Different Formulations of Povidone-Iodine, Chlorhexidine-Gluconate and Chlorxylenol in Caprine Model: A Comparative Study

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Abstract | One of the major causes of surgical site infection (SSI) is an inappropriate surgical site preparation that leads to postoperative complications. Although preoperative skin preparation is a standard surgical practice to prevent SSI, the choice of skin disinfectants and methods of skin preparation is still aberrant in veterinary practice. The study was, thus, conducted to standardize pre-surgical skin preparation with various formulations of Chlorhexidine gluconate, Chlorxylenol, and Povidone-iodine (PI) in goat. Seven surgical fields were prepared for evaluating seven formulations of these antiseptics. The bacterial swabs collected at different stages of skin preparations were transferred in nutrient broth and cultured in Plate Count Agar for counting of Colony Forming Unit (CFU). Results revealed that Chlorxylenol was less efficacious than PI and Chlorhexidine gluconate when mean CFU was counted at different stages of surgical field preparation. Soap water scrubbing and ethanol spray followed by aqueous Chlorhexidine gluconate painting eliminated 100% bacterial load and kept the site aseptic for 60 min. On the other hand, alcoholic Chlorhexidine gluconate completely removed bacterial burden from the skin and maintained 90 min long aseptic condition of the operation site. Based on our findings, alcoholic Chlorhexidine gluconate was the best in producing an aseptic surgical field for a longer duration. Our findings recommend robust scrubbing of the surgical site with detergent or soap water followed by 70% ethanol or hexisol spray and then painting the site with antiseptic preferably with alcoholic Chlorhexidine gluconate solution to generate aseptic surgical site which is very basic to prevent SSI and postoperative complications.

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1. Introduction

Careful surgical site preparation of the patients is one of the most critical skills that operating room professionals perform with patience. The objectives of surgical field preparation are to lessen the infection chance by diminishing the quantity of normal skin flora present and hindering the rapid re-development of microorganisms during surgeries (Fossum, 2007). A few microorganisms are innocuous on the surface of the skin but when they get into an entry point they can cause post-surgical infection (Wilson, 2013). Surgical site infections remain a serious complication in both human and veterinary surgery. The estimated

post-operative wound infection rate in small animal surgery ranges from 2-5.5% (Brown et al., 1997). There are a few wellsprings of tainting, for example, the patient's skin, the surgical team, the OT or emergency clinic condition, and surgical instruments or appliances. Obviously, the skin preparation of animals is more challenging compared to human patients. The thick hair coat, the inconsistency of bathing, and the more defiled condition of animals may challenge the efficacy of surgical field preparation that functions admirably in human. Inadequate or inappropriate preparation of surgical site is one of the most important cause of postoperative complications especially due to ubiquitously distributed *Staphylococcus aureus* and few other pathogenic bacterial species in food and pet animals (Alam et al., 2005; Tamanna et al., 2020).

Topical antiseptics and disinfectants are used for pre-operative skin preparation that can minimize potential infection by reducing endogenous skin microflora. Povidone-iodine, Chlorxylenol and Chlorhexidine gluconate (CHG) are common antiseptics in veterinary and human hospitals (Lambrechts et al., 2004). Alcohol-based solutions that contain CHG or iodophors have sustained and durable antimicrobial activity that lasts long after alcohol evaporation (Uppal et al., 2017). Chlorxylenol is also important antiseptic and disinfectant which is used for skin disinfection and cleaning surgical instruments.

The preparation of the surgical site generally involves a series of steps which include the clipping of hair, the removal of dirt and oils, and the removal or reduction of microbes (Bhavan et al., 2009). Each of these steps can be carried out in a few different ways and has been widely concentrated, but precise conclusions on the best method for preparing patients, especially in field level veterinary practice, have yet to be established. The study, however, was to determine whether skin asepsis immediately before surgical site incision reduced skin microbial burden and to standardize the method of pre-surgical skin preparation with various formulations of commercially available antiseptics such as Povidone-iodine, Chlorxylenol, and Chlorhexidine gluconate+cetrimide.

2. Materials and Methods

2.1 Experimental animals

Seven healthy goats of 10-12 months old weighing 8-10 kg and irrespective of sex were used for this experiment. The animals were purchased from the local market and kept in quarantine for two weeks. They were housed in a well-ventilated, wooden floor shelter with access to food and water *ad libitum*. The animals were vaccinated with PPR (PPR vaccine@ LRI, Bangladesh) and dewormed with anthelmintic (A-Mectin plus®, The Acme Laboratories, Bangladesh, Ltd).

2.2 Antiseptics

(i) Povidone Iodine 10% (Viodin®, Square Pharmaceuticals Ltd, Bangladesh), (ii) Hexisol (Hexisol® hand Rub, ACI Ltd., Dhaka, Bangladesh), (iii) Ethyl Alcohol – (Ethanol®, Merck, Germany) (iv) Chlorhexidine Gluconate 0.3% (CHG) with Cetrimide 3.0% (Savlon®, ACI Ltd., Dhaka, Bangladesh) (v) Chloxylenol 4.8% (Dettol®, Reckitt Benckiser Bangladesh Ltd.).

2.3 Animal preparation, antiseptic applications and bacterial sampling

Our procedure strictly followed the aseptic rules and regulations to collect the bacterial sample. The lateral abdominal wall of each animal was used as the experimental field. The animals were properly restrained and a sterile hair clipper was used to shave the site. To clean the surgical site, a 1-2 minute robust scrubbing with sterile gauze soaked with alkaline soap water was performed. Sterile cotton buds have been used for bacterial sampling from the experimental field at different stages of preparation. The cotton buds were quickly transferred into safe lock Eppendorf tubes containing nutrient broth. Bacterial sampling from diversely prepared surgical fields were as follows: Field-A: Collecting skin swab immediately after shaving and at 5 min, 30 min, 60 min, 90 min of post-painting with PI. Field-B: Collecting samples after shaving, scrubbing with soap water, hexisol painting, and then at 5 min, 30 min, 60 min, 90 min of post-painting with PI. Field-C: Collecting samples after shaving, scrubbing with soap water, spraying with 70% ethanol, and then at 5 min, 30 min, 60 min, 90 min of post-painting with PI. Field-D: Collecting swab after shaving, scrubbing with soap water, and after spraying with 70% ethanol and then at 5 min, 30 min, 60 min, 90 min of post-painting with alcoholic Savlon® solution. Field-E: Collecting swab after shaving, scrubbing with soap water and after spraying with 70% ethanol and then at 5 min, 30 min, 60 min, 90 min of post-painting with aqueous Savlon® solution. Field-F: Collecting swab after shaving, scrubbing with soap water and

after spraying with 70% Ethanol and then at 5 min, 30 min, 60 min, 90 min of post-painting painting with alcoholic Dettol® solution. Field-G: Collecting swab after shaving, scrubbing with soap water and after spraying with 70% Ethanol and then at 5 min, 30 min, 60 min, 90 min of post-painting painting with aqueous Dettol® solution.

2.4 Culture of bacteria and determination of bacterial load

The bacterial load was quantified by determination of total viable count (TVC). For this purpose, the collected samples were processed according to the technique described by Jaman et al., (2018) with minor modifications. Briefly, the collected samples were subjected to 10 fold dilutions and from each dilutions 100 µl was spread onto plate count agar (PCA) followed by incubation at 37 °C for overnight to develop bacterial colony.

The number of bacterial colonies were counted, calculated and expressed and as Colony Forming Units (CFU)/ml. Each experiment was repeated for successive three times.

The CFU/mL was calculated using the formula:

$$\text{CFU/mL} = \frac{(\text{No. of colonies x dilution factor})}{\text{volume of culture plate}} \times 10$$

2.5 Statistical analysis

All the data were expressed as Mean ± SE (Standard Error). To compare data among groups one-way ANOVA (Analysis of variance) factor analysis was performed using Statistical Package for the Social Sciences (SPSS) version 20.0. Probability P<0.05 was considered as statistically significant.

3. Results

3.1 Mean bacterial count on the surgical site prepared with PI only

The mean bacterial count on skin before and after application of 10% PI is presented in Figure 1. Here, immediate after shaving, the mean bacterial count was $11.8\times10^5\pm41633.31$ CFU/mL. After Five minutes of PI application, the bacterial count was significantly (P<0.05) reduced to $10.8\times10^3\pm600.92$ CFU/mL. After 30 min of PI scrubbing, the bacterial colony increased in number ($41.6\times10^3\pm4409.59^a$ CFU/mL) and the trend continued until the end of the experiment.

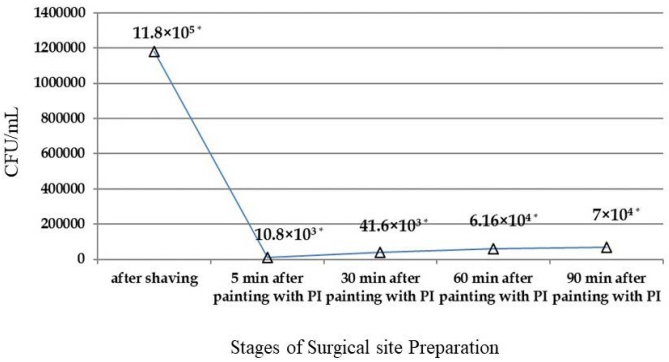

Figure 1: Mean bacterial colony counts at different stages of preoperative preparation with povidone-iodine. Results are representative of three independent experiments.

3.2 Mean bacterial count on surgical field prepared with soap water, hexisol and PI

We have evaluated soap water and hexisol scrubbing before PI painting on reducing the bacterial load from the surgical site. After clipping and shaving, the mean bacterial load was $19.3\times10^5\pm56862.41$ CFU/mL and after soap water and hexisol scrub, the bacterial colonies was significantly reduced to $1.3\text{x}10^5\pm11547.01$ and $1.2\times10^5\pm11547.01$ CFU/mL respectively. Finally PI caused complete elimination bacteria from the surgical site keeping the field aseptic up to 30 minutes. Then bacteria started to regrow and increased in number over time. The detailed results have been exhibited in Figure 2.

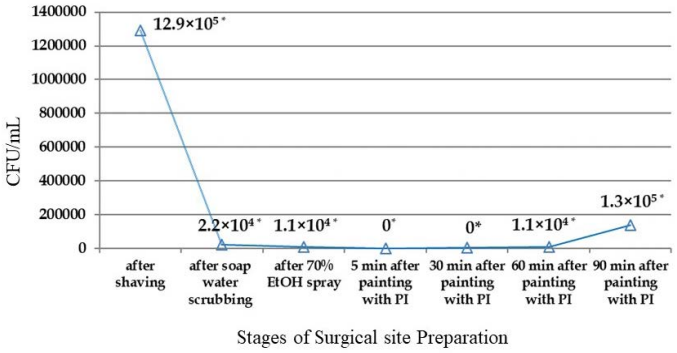

Figure 2: Mean bacterial colony counts at different stages of preoperative preparation with soap water, hexisol, and PI scrubbing. Results are representative of three independent experiments.

3.3 Effects of soap water and 70% ethanol scrub accompanied by PI mopping on minimizing bacterial load from surgical site

The efficacy of soap water, 70% ethyl alcohol followed by PI on diminishing the bacterial load from the surgical field was evaluated. We have found that immediate after shaving the mean bacterial load was

$12.9\times10^5\pm65591.12$ CFU/mL and after a robust soap water scrubbing followed by a 70% ethanol spray, bacterial counts were drastically reduced to $22.3\text{x}10^3\pm1452.97$ and $10.6\text{x}10^3\pm666.67$ CFU/mL respectively (Figure 3). After mopping with PI, bacteria were totally eliminated from the skin surface. This effect was continued up to 60 mins. After 60 mins of PI application, bacteria started to regrow and increased in number progressively.

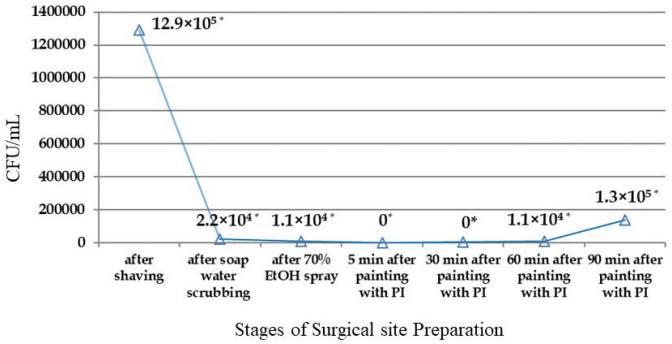

Figure 3: Mean bacterial colony counts at different stages of preoperative preparation with soap water, 70% ethanol, and PI scrubbing. Results are representative of three independent experiments.

3.4 Effect of 70% ethyl alcohol and aqueous CHG scrubbing on reducing the bacterial load from the surgical site

In this protocol, immediately after shaving mean bacterial load was $12.5\times10^5\pm28867.51$ CFU/mL and then after soap water scrubbing and 70% Ethyl alcohol spray, the bacterial colonies were reduced significantly ($P<0.05$) to $11.7\text{x}10^3\pm6009.25$ CFU/mL and $3.3\times10^3\pm3333$ CFU/mL. Subsequently after aqueous CHG solution application bacteria was totally eliminated and this effect was continued up to 60 minutes. After 90 min, we experienced reappearance and gradual elevation of the bacterial load (Figure 4).

3.5 Effects of soap water and 70% ethanol followed alcoholic CHG solution scrubbing on reducing the bacterial load from surgical site

We have evaluated the efficacy of alcoholic CHG solution after soap water scrubbing and 70% ethyl alcohol spray. Here immediate after shaving the mean bacterial count was $13.1\times10^5\pm46144.7$ CFU/mL which reduced to $12.3\text{x}10^3\pm18559.21$ CFU/mL after soap water scrubbing. Following 70% ethyl alcohol spray, 99.7% bacteria were eliminated from the surgical site and after alcoholic CHG solution application, bacteria were completely removed. This effect was continued up to 90 minutes which was the longest duration of keeping aseptic surgical field among all the protocol we tested. The mean bacterial count at different stages of skin preparation is presented in Figure 5.

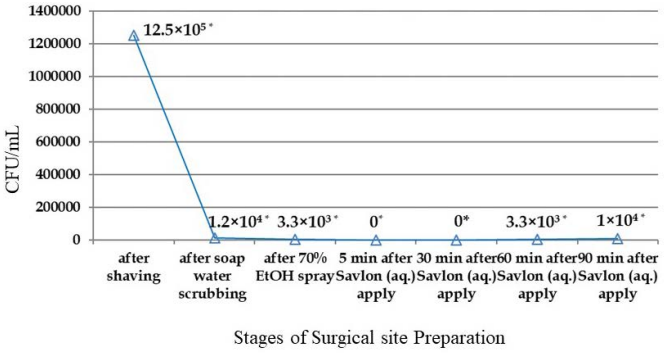

Figure 4: Mean bacterial colony counts at different stages of preoperative preparation with soap water, 70% ethanol, and aqueous CHG (Savlon®) scrubbing. Results are representative of three independent experiments.

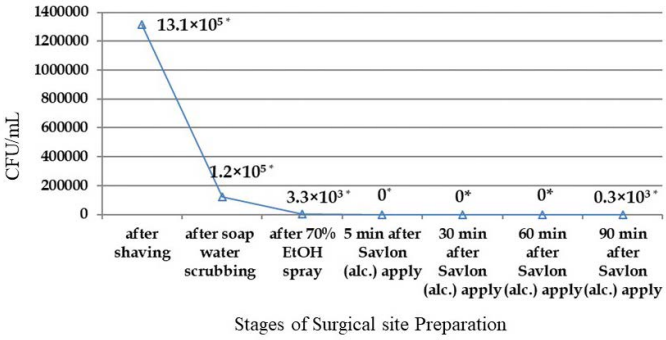

Figure 5: Mean bacterial colony counts at different stages of preoperative preparation with soap water, 70% ethanol, and alcoholic CHG (Savlon®) scrubbing. Results are representative of three independent experiments.

3.6 Determination of mean bacterial load on surgical site before and after soap water, ethanol and aqueous chlorxylenol solution scrubbing

During this procedure immediately after clipping and shaving the mean bacterial load was $13.9\times10^5\pm51385.26$ CFU/mL (Figure 6). After soap water and 70% ethanol scrubbing, bacterial count decreased to $2.1\times10^5\pm21858.13$ CFU/mL and $90\times10^3\pm5773.5$ CFU/mL respectively that means soap water scrubbing and ethanol spray can reduce 94% bacterial burden before antiseptics applications. Once aqueous Dettol® solution was applied, nearly 99% bacteria were eliminated from the surgical site. This method did not eliminate the bacteria completely from surgical field.

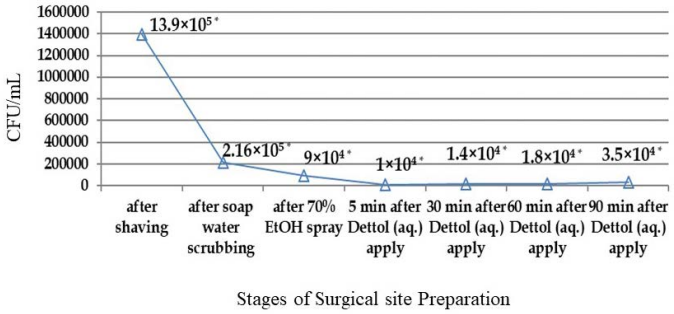

Figure 6: Mean bacterial colony counts at different stages of preoperative preparation with soap water, 70% ethanol, and aqueous Chlorxylenol (Dettol®) scrubbing. Results are representative of three independent experiments.

3.7 Mean bacterial count on the surgical field prepared by soap water, ethyl alcohol and alcoholic chlorxylenol (Dettol® (alc.) solution scrubbing

In this method, we have performed soap water and 70% ethyl alcohol scrubbing followed by painting the site with Dettol® (alc.) solution. After clipping and shaving the mean bacterial count was $15.1\times10^5\pm48529.49$ CFU/mL and this was reduced to $3.7\times10^5\pm110746.5$ CFU/mL and $10\times10^3\pm 666.67$ CFU/mL after soap water scrubbing and 70% ethyl alcohol spray respectively. Thereafter, alcoholic Dettol® solution completely eliminated bacteria from the site. After 30 minutes, bacteria became evident and increased in number over time (Figure 7).

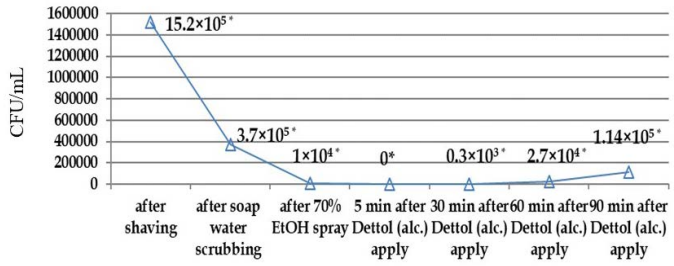

Figure 7: Mean bacterial colony counts at different stages of preoperative preparation with soap water, 70% ethanol, and alcoholic Chlorxylenol (Dettol®) scrubbing. Results are representative of three independent experiments.

3.8 Comparative effectiveness of PI, CHG, and Chlorxylenol in reducing the bacterial colonies from the surgical site

Figure 8 represents the assessment among the formulations of antiseptics and the methods employed in minimizing the mean bacterial load from the operation field. In this comparative analysis, we found that the alcoholic solution of CHG + cetrimide (Savlon®) performed better in reducing bacterial load and maintaining aseptic field for longer duration than other formulations.

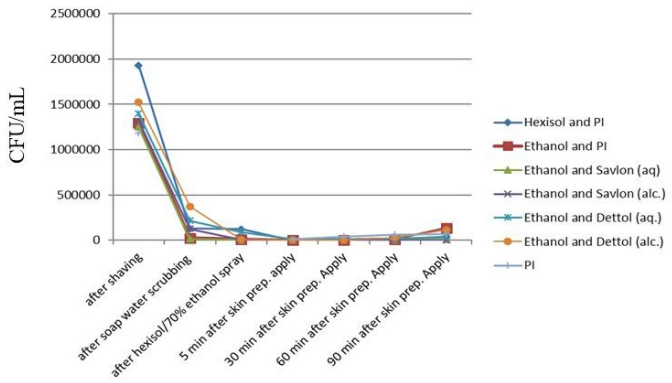

Figure 8: Comparative effectiveness of PI, CHG, and Chlorxylenol following different techniques in reducing the bacterial load from the surgical site.

Discussions

Surgical site incision is a typical practice in the intrusive medical procedure which breaks in the cohesion of the skin and subcutaneous tissues (Miah et al., 2017; Mohiuddin et al., 2018). This procedure doesn't just interfere with the defensive barrier of the patient; it likewise permits passage, defilement, multiplication, and expansion of contaminating microorganisms. Catastrophic fate culminating in post-surgical complications can be the aftereffect of infection of surgical sites by contaminating organisms due to poor surgical sites preparation or a total lack of surgical sepsis invasive procedure (Melekwea et al., 2018). Effective pre-surgical preparatory protocols, with adequate infection control practices are fundamental for the prevention of SSIs (Abbas and Pittet, 2016).

We were intended to standardize the surgical site preparation method especially for veterinary practice, as this process is still anomalous which happens in many veterinary hospitals. In our study, it was clear that initially all the experimental field had a huge bacterial load prior to scrubbing with soap water, hexisol or 70% ethanol. After strong scrubbing with soap water, followed by either hexisol or ethanol spray, there was a significant reduction in skin bacterial load. Our results from PI showed a significant reduction of bacterial colony count to levels minimal enough to prevent surgical site infection in goats and maintained

an around 60 mins long asepic condition of surgical site. Similar results on the efficacy of PI have been reported before by other researchers (Park et al., 2017; Uppal et al., 2017). This indicates that this antiseptic is also effective and can be used as scrubbing agent as the mean colony count was less than 10^5, which is the minimal count for prevention of wound infection.

On the other hand, both aqueous and alcoholic CHG+ Cetrimide provided dramatic effect against skin microflora when soap water scrubbing and 70% ethanol were applied prion to CHG +cetrimide scrubbing. We have shown skin prepared with CHG +cetrimide remarkably reduced the mean CFU allowing 90 mins long aseptic condition of surgical site. The results of this study were similar to the previous findings where chlorhexidine-based antiseptic preparations were more effective than iodine-containing solutions in reducing the skin bacterial load from the surgical field (Bibbo et al., 2005; Nishimura, 2006; Yakubu et al., 2010). The clinical protection and antiseptic efficacy provided by CHG +cetrimide may be related to its fast acting capabilities, persistence activity irrespective of the presence of contaminants and overt exposure to body fluids and also its residual effect on tissues (Abbas and Petal, 2016).

Chlorxylenol solution (aqueous and alcoholic) also showed great reduction of bacterial count to minimum level that cannot allow surgical infection. We did not find complete elimination of bacteria from surgical site prepared with aqueous formulation of Chlorxylenol which is similar to the report of Yakubu et al. (2010).

We have found the regrowth of bacteria after antiseptic scrubbing. The stock solution of all the antiseptics has been reduced to a level of bacteriostatic effects rather than bactericidal action. This may be the reason behind regrowth and gradual increase in number of bacteria.

Conclusions and Recommendations

It can be concluded that CHG + cetrimide, Chlorxylenol and PI can be used comfortably as skin antiseptic solution with a wide margin of safety during surgical procedures. CHG +cetrimide, however, is more effective at the surgical site in dramatic reduction of bacterial colonies, holding zero bacterial loads for a longer period. Our findings suggest that the operating field should be cleaned and scrubbed robustly with soap water, hexisol, or ethanol before applying these skin antiseptics to gain a site of clean and less bacterial colonies.

Authors' Contributions

This work was carried out in collaboration between all authors. Md. Al-Amin Tan conducted the research. Mst. Antora Akter and Md. Sabuj Rahman assisted in sampling and edited manuscript. Marzia Rahman performed bacteriological work. Md. Mahmudul Alam designed the study, supervised the research, written the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The author declares that there is no conflict of interest in publishing this paper.

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