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Research article

Evaluating efficacy of preoperative skin preparation with 2% chlorhexidine gluconate and 70% alcohol for reducing bacterial bioburden in dogs

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Abstract

Importance of the work: Optimized antisepsis and protocols are essential for improving veterinary surgical outcomes and preventing surgical site infections (SSIs).

Objective: To assess the effectiveness of 2% chlorhexidine gluconate and 70% alcohol (CHG-Alc) in reducing the bacterial bioburden, including multidrug-resistant strains, in canine surgical patients.

Materials and Methods: A sample of 10 client-owned dogs was involved in the proposed standardized skin preparation protocol. Following an initial skin cleaning step, CHG-Alc was sprayed onto the skin and left in contact for 3 min. Then, the excess solution was gently removed. Bacterial cultures were collected at three time points: prior to antiseptic application (the first), immediately after the 3-min contact period and removal of the solution (the second) and 60 min after application (the third). Samples were processed to quantify bacterial load, identify bacterial species and assess antimicrobial resistance.

Results: Pre-antisepsis, *Staphylococcus pseudintermedius* was the most frequently isolated bacterium, including the multidrug-resistant strains. Bacterial counts decreased significantly by over 99% at the second sampling time ($p = 0.006$), with complete absence of growth (0 CFU/mL) at the third sampling time ($p = 0.006$).

Main Finding: CHG-Alc was highly effective for canine preoperative skin preparation, significantly reducing bacterial counts and preventing reinfection. There should be strong support for its use in veterinary preoperative protocols to minimize the risk of surgical site infections.

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Introduction

Veterinary surgeons face unique challenges in preparing animal patients for surgery because animals present with distinctive anatomical and behavioral characteristics that complicate surgical site preparation, including dense hair coat, increased exposure to environmental contaminants and natural grooming behavior that may introduce additional pathogens (Bhavan and Warren, 2009). These factors necessitate specialized approaches to achieve adequate surgical site asepsis. Despite advances in modern surgical techniques and antimicrobial strategies, surgical site infections (SSIs) remain a major concern, impacting patient recovery and surgical outcomes (Jolivet and Lucet, 2019; Seidelman et al., 2023). Most SSIs originate from endogenous skin microflora (Singh et al., 2013). *Staphylococcus* spp. is the most isolated bacterial genus from SSIs in veterinary medicine, with *Staphylococcus pseudintermedius* being particularly prevalent in canine infections (Brown, 2012; Verwilghen and Singh, 2015). This species is a commensal organism of the normal canine skin that can opportunistically cause infection, especially when skin integrity is compromised (Campbell et al., 2013). *S. pseudintermedius* has numerous virulence factors that can complicate treatment, including adhesins, toxins and biofilm formation, that facilitate colonization and enhance resistance to antimicrobials and host immune responses. (Campbell et al., 2013; Singh et al., 2013). In addition, the emergence of multidrug resistant (MDR) and methicillin-resistant *S. pseudintermedius* (MRSP), poses major challenges in managing SSIs (Singh et al., 2013; Verwilghen and Singh, 2015). Therefore, effective preoperative skin preparation with proper protocols and appropriate antiseptic agents are critical components in reducing bacterial contamination and preventing SSIs (Huitson 2023; Nye and Thieman, 2024).

Generally, routine preoperative skin preparation in veterinary patients involves hair removal and initial cleansing to remove debris, followed by the application of antiseptic solutions on the surgical site (Bhavan and Warren, 2009; Pelosi, 2018). Among available options, the combination of chlorhexidine gluconate (CHG) and alcohol has emerged as a preferred choice due to their complementary mechanisms of action. CHG provides broad-spectrum antimicrobial activity through bacterial cell membrane disruption and is particularly effective against Gram-positive bacteria, including *Staphylococcus* species, as well as Gram-negative bacteria, yeasts and some viruses (Lim and Kam, 2008). The substantivity of CHG allows it to bind to the keratin layer of the skin, providing ongoing antimicrobial activity (Lim and Kam, 2008; Nye and Thieman, 2024). In addition, alcohol delivers rapid initial killing through protein denaturation and lipid membrane disruption (Ali et al., 2000). This immediate action by alcohol, coupled with the sustained action of CHG, creates a combined effect that addresses both rapid initial bacterial reduction and prolonged inhibition of microbial growth (Crabtree et al., 2000; Nye and Thieman, 2024).

While CHG and alcohol preparations are commonly used in clinical practice, a consistent standard protocol for their application has not been established. Studies in canine surgical preparation have investigated various protocols, ranging from sequential application of 2–4% CHG followed by alcohol (Gibson et al., 1997; Belo et al., 2018) to more complex multiple applications using the combination of CHG with alcohol or saline (Lambrechts et al., 2004; Andrade et al., 2016). However, a CHG concentration exceeding 2% has not been shown to decrease SSI and has been associated with an allergic reaction (Hasegawa et al., 2022). Frequently, a minimum contact time of 3 min is recommended in clinical antiseptic studies (Stubbs et al., 1996; Lambrechts et al., 2004; Boonwittaya et al., 2024) to ensure optimal bacterial reduction. Specifically, chlorhexidine has been recommended to remain on the skin for at least 3 min before surgery to maximize its bactericidal efficacy; however, this contact time may be reduced when combined with alcohol due to their synergistic bactericidal effects (Curtis, 2021; Hasegawa et al., 2022; Nye and Thieman, 2024). Despite these findings, further research is necessary to establish the optimal skin asepsis protocol and to evaluate the efficacy of these antiseptics in combination for bacterial decontamination, as the selection of antiseptic agents and their application methods greatly impacts surgical success rates and the prevention of surgical site infections (SSIs). Therefore, the current study aimed to evaluate the efficacy of preoperative skin preparation protocols and the combination of 2% chlorhexidine gluconate and 70% alcohol (CHG-Alc) in reducing bacterial bioburden, including multidrug-resistant strains in canine surgical patients.

Materials and Methods

Animals

The study was conducted on 10 client-owned dogs (median age [range]: 27 mth [7–94 mth], consisting of 3 males and

7 females; median weight [range]: 19.2 kg [14.2–31.7 kg]) visiting the Kasetsart University Veterinary Teaching Hospital, Bangkok, Thailand. All dogs were fully vaccinated, clinically healthy and had normal hematological and serum biochemical profiles. They were free of visible skin lesions, as well as any history of concurrent diseases or injuries. None of the dogs had received antibiotics within 48 hr prior to the study. Food was withheld for 6 hr and water for 4 hr before performing general anesthesia.

Study design

All dogs were pre-medicated intravascularly (IV) using 0.1–0.3 mg/kg midazolam (Midazolam-hameln 0.5%; Siam Bioscience Co. Ltd.; Nonthaburi, Thailand). Then, anesthesia was induced using propofol (Troypofol 1% w/v; Troikaa Pharmaceuticals Ltd.; Uttarakhand, India) at a dosage of 1–4 mg/kg IV and maintained with sevoflurane (SEVO 100%; Singapore Pharmawealth Lifesciences Inc.; Laguna, the Philippines) combined with oxygen. No antibiotics were administered at any point during the study period (from the initiation of anesthesia to the final sample collection). The entire ventral abdomen, including the preputial area in male dogs, was clipped using sterilized electric clippers and sterilized blades. Preputial or vaginal cleaning was performed and urine was drained following the hospital's standard protocol (Boonwittaya et al., 2024).

The skin was cleaned with a neutral detergent using sterile sponges soaked in the cleaning solution, wiping in a back-and-forth motion starting at the midline and moving outward (Curtis, 2021). This was followed by rinsing the area with sterile water. The procedure was repeated at least three times to ensure the removal of dirt and hair. Staff wore surgical caps, masks and gloves during this pre-antiseptic skin washing. Once the skin had been dried using a sterile paper towel, the staff changed to new sterile gloves and collected the pre-antiseptic culture sample (the first) using a modified swabbing method. Three non-overlapping but adjacent 10 × 10 cm areas within the ventral abdominal region were used for sequential sampling to avoid mechanical disruption and interference from residual antiseptic. A sterile cotton swab (Sterile Cotton Swab; Thai Gauze Co. Ltd.; Bangkok, Thailand) was gently rolled over each area, delineated using a sterile acrylic frame placed on one lateral side of the ventral abdomen (Fig. 1).

The entire prepared area was thoroughly saturated with a spray solution containing 2% chlorhexidine gluconate and 70% ethyl alcohol (Prepskin-C; Medicpharma Co. Ltd.; Samut Sakhon, Thailand). The solution was left on the skin for 3 min, after which any excess was gently removed by absorption into a dry, sterile paper towel. Next, the post-antiseptic culture sample (the second) was collected from the opposite side of the ventrolateral abdomen. Draping of the dog was performed immediately after the second sample collection. The final culture sample (the third) was obtained from the ventral midline, ensuring no overlap with previous sampling sites, 60 min after the antiseptic application. Each bacterial collection used a new pair of sterile gloves and sterilized frame.

Microbiological assessment

All sampling was conducted by a single operator. Each culture swab was placed in a test tube containing 5 mL of buffered peptone water and transported immediately to the Microbiology Laboratory at the Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand for bacterial cultivation. The samples were resuspended by vortex mixing and a tenfold serial dilution of each sample was prepared from 10 to 10^{-3}. Then, 100 µL of each dilution was spread onto blood agar and MacConkey agar plates. All plates were incubated at 35 ± 2°C for 48 hr. Bacterial colony-forming units (CFUs) were counted and numbers calculated based on the dilution series. The results were presented as colony-forming units per milliliter. Distinct colonies were isolated; bacterial species in the purified isolates were identified using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) (MALDI Biotyper; Bruker Daltonics; Bremen, Germany). Antimicrobial resistance was assessed for 10 different antibiotics using the disk diffusion method on Mueller-Hinton agar.

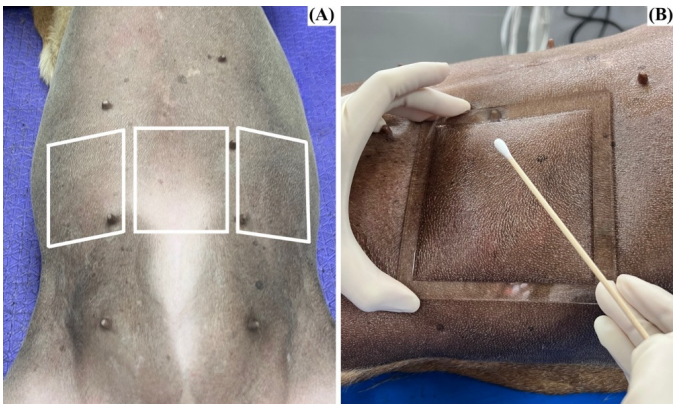

Fig. 1 Designated skin areas for bacterial culture sampling: (A) three separate sites in ventral abdominal region used for sample collection: one lateral side for pre-antiseptic sampling, opposite lateral side for second sampling and ventral midline for third sampling; (B) sampling area delineated using a sterile 10 × 10 cm acrylic frame during swab collection.

The antimicrobial susceptibility individual discs (Oxoid™; Thermo Fisher Scientific Inc.; Basingstoke, UK) consisted of amikacin (30 µg), amoxicillin-clavulanic acid (20 + 10 µg), azithromycin (15 µg), cephalexin (30 µg), ceftriaxone (30 µg), clindamycin (2 µg), imipenem (10 µg), doxycycline (30 µg), trimethoprim-sulfamethoxazole (1.25 + 23.75 µg) and enrofloxacin (5 µg). For the *Staphylococcus* spp. isolates, additional discs of cefoxitin (30 µg) and oxacillin (1 µg) were used to assess methicillin resistance. Guidelines (Clinical and Laboratory Standards Institute, 2024a, b) were used to interpret the antimicrobial-resistant results, with intermediate susceptibility also classified as resistant. Isolates exhibiting resistance to at least three distinct classes of antibiotics were classified as multidrug-resistant (MDR), according to Gandolfi-Decristophoris et al. (2013).

Statistical analysis

The G*Power software version 3.1.9.6 (Faul et al., 2009) was used to estimate the required sample size to detect differences in CFU levels between pre-antiseptic and post-antiseptic sampling times, using a beta value of 0.8 and an alpha value of 0.05 (two-tailed test). The statistical analysis was performed using the R Statistical Software version 4.3.2 (R Core Team, 2024). The normal distribution was analyzed using the Shapiro-Wilk test and the results were presented based on non-parametric methods for analysis. The CFU levels at different sampling time points were compared using the Wilcoxon signed-rank test. Statistical significance was set at $p < 0.05$.

Ethics statements

This study was approved by the Kasetsart University Institutional Animal Care and Use Committee (Approval ID #ACKU66-VET-004), Bangkok, Thailand.

Results

Bacterial culture and quantification

All samples collected during the pre-antiseptic sampling period exhibited positive bacterial growth after incubation. However, at the second sampling time, only 20% (2/10) of the samples showed bacterial growth, while there was no bacterial growth in any sample from the third collection time.

The median bacterial quantification at the first sampling time (pre-asepsis) was 81,500 CFU/mL (interquartile range (IQR) [range] = 122,769 [2,975–165,000]). The median bacterial quantification was 0 CFU/mL at both the second sampling time (IQR [range] = 0 [0–25]) and the third sampling time (IQR [range] = 0 [0–0]). There was a significant reduction in the bacterial bioburden between the first and second sampling time points, as well as between the first and third sampling time points ($p = 0.006$ for both comparisons), as illustrated in Fig. 2.

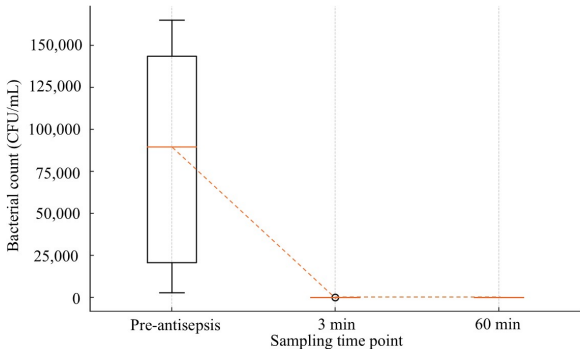

Fig. 2 Reduction of median bacterial counts before and after 2% chlorhexidine gluconate with 70% alcohol application, where upper bar indicates maximum value, lower bar indicates minimum value and horizontal red lines is median value and CFU = colony-forming units

Bacterial species identification

The most frequently isolated organism at the pre-antiseptic time point was *S. pseudintermedius*, followed by *Macrococcus canis* and other commensals such as *Micrococcus luteus*, *Bacillus cereus* and *Staphylococcus chromogenes*. The prevalence levels of all isolated bacteria are detailed in Table 1. At the second sampling time, *Staphylococcus epidermidis* (25 CFU/mL) and *S. pseudintermedius* (25 CFU/mL) were isolated from two different dogs.

Antimicrobial resistance profiles

Among the pre-antiseptic isolates, one isolate of *S. pseudintermedius* in one dog was MDR, exhibiting resistance to azithromycin, clindamycin, doxycycline and enrofloxacin, with the highest bacterial count (163,000 CFU/mL). Furthermore, one isolate of *Micrococcus luteus* from another dog was MDR, exhibiting resistance to azithromycin, cephalexin, ceftriaxone, clindamycin and trimethoprim-sulfamethoxazole, but with a lower bacterial count (2,250 CFU/mL). No MRSP was detected in any samples across all sampling times. The profiles of antibiotic resistance among all bacterial isolates are summarized in Table 2.

Table 1 Prevalence of bacterial isolates at pre-antiseptic sampling time (colony-forming units per milliliter)

Case No.	*Staphylococcus pseudintermedius*	*Staphylococcus chromogenes*	*Macrococcus canis*	*Rothia amarae*	*Priestia flexa*	*Bacillus cereus*	*Micrococcus luteus*
1	71,750	250	0	0	0	0	0
2	12,500	12,500	140,000	0	0	0	0
3	50,000	0	0	300	0	0	0
4	139,250	0	0	0	1,250	0	0
5	144,750	0	0	0	0	0	0
6	8,250	0	0	0	0	0	0
7	163,000	0	0	0	0	0	0
8	88,250	0	0	0	0	500	2,250
9	0	0	0	2,975	0	0	0
10	11,125	0	0	0	0	0	0

Table 2 Numbers of isolates of antibiotic-resistant bacteria from pre-antiseptic sampling time

Bacterial strain	Number of isolate(s) showing antibiotic resistance									
	AK	AMC	ATH	CL	CRO	CD	DXT	IMI	STX	ENF
Staphylococcus pseudintermedius (*n*=9)	1		1			1	1			1
Bacillus cereus (*n*=1)		1			1					
Micrococcus luteus (*n*=1)			1	1	1	1			1	
Rothia amarae (*n*=2)			2			1				

n = total number of isolates; AMK = amikacin, AMC = amoxicillin-clavulanic acid, ATH = azithromycin, CL = cephalexin, CRO = ceftriaxone, CD = clindamycin, IMI = imipenem, DXT = doxycycline, STS = trimethoprim-sulfamethoxazole and ENF = enrofloxacin.

Discussion

Surgical site infections remain a major concern in veterinary medicine, with the skin's resident microflora serving as a primary source of contamination (Singh et al., 2013). This study aimed to evaluate the effectiveness of preoperative skin preparation using CHG-Alc to reduce bacterial bioburdens in canine patients. Based on the current results, there was significant bacterial reduction following antiseptic application, with effects sustained throughout the observation period. The numbers of resident bacteria on the canine skin varies case by case, typically in the range 100–1,000 organism/cm^2 of aerobic bacteria (Campbell et al., 2013). In the current study, the pre-antisepsis bacterial loads were in the range 2,975–165,000 CFU/mL, based on samples collected from a 100 cm^2 skin area. The wide range of pre-antiseptic bacterial counts observed in this study likely reflected individual variability in canine skin microbiota, which can be influenced by multiple factors, including individual differences in grooming behavior, skin physiology, coat density, environmental exposure and microbial colonization patterns (Campbell et al., 2013). These results highlighted the challenges of achieving effective skin decontamination in veterinary settings, where multiple factors contribute to higher microbial counts in canine patients compared to humans (Bhavan and Warren, 2009). This is particularly important, as bacterial loads exceeding 1×10^5 CFU/mL are strongly associated with increased SSI risks (Mangram et al., 1999; Kil and Swanson, 2011).

A sampling area of 10 cm × 10 cm was used in this study, similar to the study by Belo et al. (2018). This size contrasts with the smaller sampling areas and the even smaller surface sampled using RODAC plates as in the other studies (Lambrechts et al., 2004.; Boonwittaya et al., 2024). The larger sampling area likely allowed for the collection of a greater bacterial load, which may explain the higher bacterial counts observed in the current study and reported by Belo et al. (2018). However, notably, cleaning efficacy diminishes over larger surface areas, potentially leading to increased bacterial recovery. In contrast, smaller sampling areas may provide less variability and better reflect localized cleaning efficacy but might underestimate the overall bacterial burden across larger regions. These differences highlight how sampling area size can greatly influence bacterial count results and the interpretation of cleaning efficacy in comparative studies. Although minor regional variation in skin flora is possible, swab samples collected from adjacent, non-overlapping areas within the ventral abdominal region to prevent disruption of the antiseptic barrier, minimize cross-contamination and ensure accurate evaluation of antiseptic efficacy at each time point. This design has been used in similar veterinary studies (Lambrechts et al., 2004; Boucher et al., 2018)

All pre-antiseptic samples produced bacterial growth, with *S. pseudintermedius* as the predominant species. This bacterium is the most common *Staphylococcus* species isolated from dogs, acting as a commensal organism on their skin and mucous membranes (Campbell et al., 2013; Singh et al., 2013). Colonization rates in healthy dogs have been reported in the range 30–70%; though only a small percentage developed infections, often triggered by underlying factors such as skin trauma (Weese, 2012). The polymicrobial environment observed in the current study, including various species, such as *Staphylococcus* spp. and *Macrococcus canis*, as well as common hair bacterial residents, such as *Bacillus* spp. and *Micrococcus* spp. *S. pseudintermedius*, is associated with various infections, including pyoderma, urinary tract infections and endocarditis and has emerged as the leading cause of canine SSI, with increasing methicillin and multidrug resistance (Perreten et al., 2010; Weese and van Duijkeren, 2010; Weese, 2012; Campbell et al., 2013; Somayaji et al., 2016). The current study identified the specific case as MDR *S. pseudintermedius* that had a notably high bacterial count (163,000 CFU/mL) at the pre-antiseptic sampling time point. However, *S. pseudintermedius* was detected at very low bacterial counts from the second sampling and did not exhibit MDR. At the third sampling time, no *S. pseudintermedius* growth was detected in any samples after incubation. *M. canis* was one of the most prevalent bacteria cultured at pre-antiseptic time, second only to *S. pseudintermedius*. *M. canis* can be isolated from both healthy dogs and from infection sites as an opportunistic pathogen, with colonization not necessarily leading to disease. However, *M. canis* strains may be MDR or methicillin resistant (Cotting et al., 2017) and can be misidentified as *Staphylococcus* spp. without advanced diagnostic methods, such as MALDI-TOF MS or genetic methods (Gobeli et al., 2017). In the current study, *M. canis* did not exhibit multidrug resistance. *Micrococcus luteus*, a normal resident of canine skin (Campbell et al., 2013), was one of the bacteria isolated from the first sampling time and was MDR. However, *M. luteus* growth was not found in samples collected at the second and the third sampling times. The presence of multiple species suggests potential synergistic relationships that could enhance bacterial survival and colonization capabilities. This is particularly relevant in veterinary practice, where some skin microbiota may persist in deeper layers, posing additional challenges for complete decontamination (Renberg, 2011). Along with the high bacterial loads and species diversity, the presence of MDR bacteria in the current pre-antiseptic samples emphasized the importance of reducing bacterial loads prior to surgery by the effective preoperative skin preparation in preventing potential surgical site infections.

Preoperative antiseptic protocols play a critical role in mitigating this risk. Currently, there is no universal standardization for preoperative skin preparation protocols in veterinary medicine. These protocols often differ based on factors, such as the type of surgical procedure, anatomical location and institutional practices (Gibson et al., 1997). Typically, the process involves a sequence of steps aimed at minimizing the microbial load on the skin. This includes hair removal and cleansing to eliminate debris, oils and dirt, followed by the application of antiseptic agents. Generally, an initial wash using a neutral, non-medicated soap is recommended prior to the application of antiseptic solutions. Then, antiseptics are applied as the final step which can be applied using various techniques, such as scrubbing, painting or spraying. Often, among available antiseptics, alcohol-based solutions are preferred due to their rapid action and ease of application (Pelosi, 2018). CHG has been used both individually followed by rinsing with saline or alcohol or in combination with other agents, demonstrating its versatility and effectiveness in veterinary settings (Gibson et al., 1997; Belo et al., 2018; Lambrechts et al., 2004). Based on the current results, a single-step process is recommended, involving the direct application of the CHG-Alc combination that is not only more time-efficient but also highly effective. Although detailed sampling steps were applied for research standardization, the core antiseptic preparation protocol (hair clipping, neutral detergent cleansing and a single application of 2% CHG with 70% alcohol) is straightforward and practical for routine clinical use. However, the use of a single application of an antiseptic in the current study likely resulted in higher bacterial counts compared to the multi-step antiseptic application protocols commonly used in clinical practice. The near-complete elimination of bacterial species observed in the post-antiseptic results highlighted the remarkable efficacy of CHG-Alc and reinforced its value as a recommended approach for preoperative skin preparation.

The use of 2% CHG was highly effective, with bacterial reduction rates exceeding 99% after application. This concentration appeared to be optimal for surgical site preparation, as supported by recent meta-analysis findings. For example, Hasegawa et al. (2022) demonstrated that while there were no significant differences in surgical site infection prevention with higher concentrations of CHG (4% versus 2%), 2% CHG was sufficient for effective antimicrobial action. Alcohol-based products are recommended in the guidelines

for preoperative skin preparation, because they provide greater efficacy and last longer than water-based agents (Hemani and Lepor, 2009; Berrios-Torres et al., 2017). Hibbard et al. (2002) presented that combining chlorhexidine and alcohol provided advantages over standalone alcohol (which was effective but had a shorter-duration antiseptic action) or aqueous chlorhexidine (which was less effective than the other two agents). The synergistic action of CHG and alcohol was clearly demonstrated in the current results, with initial bacterial counts being effectively reduced and maintained at minimal levels throughout the study period. CHG has the ability to bind to the stratum corneum and provide residual antimicrobial activity (Lim and Kam, 2008), while alcohol provides rapid bactericidal action, ensuring an immediate reduction in bacterial load (Nye and Thieman, 2024). Notably, at the second sampling time point, there were two samples that had minimal bacterial growth (25 CFU/mL each).

The complete absence of bacterial growth in all samples at 60 min post-application suggested the sustained antimicrobial activity of the CHG-Alc combination, likely due to a residual effect of CHG on the skin surface. Notably, MRSP was absent in the current study population, though this may have been influenced by the sample size and the geographical location of the study. Nevertheless, the significant reduction in bacterial counts (including MDR strains) suggested that the developed CHG-alcohol protocol was highly effective and had potential utility in broader clinical applications. Although the current study confirmed sustained bacterial suppression up to 60 min post-application, future investigations with extended observation periods (such as 4 hr or 6 hr) are recommended to fully assess the residual effect of CHG-Alc combinations, particularly the long-lasting activity of chlorhexidine on the skin surface

The developed protocol used a 3 min contact time for the CHG-Alc solution, which proved effective in achieving significant bacterial reduction. This duration was consistent with the recent study by Boonwittaya et al. (2024), demonstrating that a 3 min contact time was a practical and effective approach in clinical settings. This timeframe strikes an optimal balance between ensuring adequate antiseptic action and maintaining efficient surgical workflow, particularly important in veterinary practice where prolonged preparation times may impact anesthesia management.

The current findings supported the adoption of standardized antiseptic protocols in veterinary surgical settings, aligning with current trends in veterinary practice regarding the choice of antiseptic solutions. The combination of 2% CHG with 70% alcohol, applied with a 3 min contact time, effectively reduced the bacterial bioburden in the cohort of canine surgical patients investigated. This protocol is both practical and efficient, making it suitable for routine clinical use while maintaining high standards of surgical site preparation. The consistent and significant bacterial reduction observed, regardless of the initial bacterial load or species diversity, highlighted the effectiveness of CHG-Alc in addressing the higher bacterial burdens frequently encountered in veterinary patients. The sample collection from non-overlapping but adjacent areas within the same region may have introduced some anatomical variability in microbial load. Future studies could consider parallel sampling from identical sites using a split-area approach to improve statistical power while minimizing antiseptic interference. Other limitations of the current study were the relatively small sample size and the focus on a single geographic location. Future research should involve larger populations across diverse regions and long-term follow-up to monitor surgical site infections, which should provide further insights into the clinical importance and broader applicability of these findings.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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