Failure to achieve asepsis following surgical skin preparation is influenced by bacterial resistance to chlorhexidine, but not skin preparation technique

Nicola Swales BSc (Hons), RVN
Small Animal Hospital, Langford Vets, Langford House, Langford, Bristol BS40 5DU, UK
Tristan Cogan BSc (Hons), PhD
School of Veterinary Science, University of Bristol, Bristol, UK

ABSTRACT: Samples were taken before and after skin preparation in 25 dogs undergoing abdominal surgery. Bacterial culture and sensitivity testing was undertaken. Eight of 25 dogs had bacteria present after preparation, of which three were prepared using the concentric circle and five using the linear method. There was no significant difference in the efficacy in achieving asepsis of either method. Four of 10 bacterial isolates remaining after surgery were resistant to chlorhexidine; two were resistant to chlorhexidine at the concentration at which it was used for skin preparation. Chlorhexidine resistance is a risk factor for the presence of bacteria post-preparation.

Nicola Swales BSc (Hons), RVN

Nicola graduated from the University of Bristol in 2015 with a degree in Veterinary Nursing and Bioveterinary Science. She started working as a dermatology nurse at University of Bristol after graduating and is involved in both clinical work and teaching of veterinary nursing and veterinary science students.
Email: nicola.swales@bristol.ac.uk

Tristan Cogan BSc (Hons), PhD

Tristan is a Senior Lecturer in Infectious Disease at the University of Bristol. After his PhD at the University of Exeter he worked on zoonotic diseases in humans for the Health Protection Agency for a number of years before moving to Bristol to study infectious diseases of animals.

Introduction

There are two commonly used methods of pre-surgical skin preparation of canine patients: the linear method, with a "back and forth" motion, and the concentric circle method, which starts in the centre, working outwards towards the limits of the surgical site.

The first stage of skin preparation in veterinary patients is hair removal. Once the hair has been removed, the primary scrub takes place. The purpose is to remove dirt, debris and transient bacteria. To do this, a combination of biocides and mechanical cleansing is needed. The vigour with which one scrubs is important because friction is needed to remove tightly adhered resident flora; however, abrasions could cause recontamination (Bowers, 2012).

Historically, the preferred scrub method has been to start at the proposed incision site and work out towards the margins of the clipped site in concentric circles (Mangram, Horan, Pearson, Silver, & Jarvis, 1999). Anecdotal evidence states that it prevents the incision site being recontaminated by marginal areas. There does not appear to be much evidence for this (Bowers, 2012) and some suggest that this method does not allow adequate penetration into fissures in the skin. Others suggest that the linear method is more efficacious (Roberts, 2013), while some say a combination should be used (Crosby, 2000).

Veterinary patients have high numbers of bacteria on the skin as, in general, they do not wash like humans, are kept in dirtier environments and have more hair follicles.

DOI: 10.1080/17415349.2017.1328994

Animals are more likely to become contaminated via the oral–faecal route and to spread bacteria during grooming.

There incidence of surgical site infections (SSIs) in both human and veterinary patients is substantial, with studies showing they occur in at least 15% of elective human patients (Dumville, McFarlane, Edwards, Lipp, & Holmes, 2004). The aim of skin preparation is to reduce the risk of SSIs by removing bacteria and inhibiting regrowth (Bowers, 2012).

The most commonly used biocides are povidone–iodine and chlorhexidine (CHX). Studies have shown CHX to be superior in reducing the amount of bacteria and incidence of SSIs (Berry, Watt, Goldacre, Thomson, & McNair, 1982; Darouiche et al., 2010; Mimoz et al., 2007; Noorani, Rabey, Walsh, & Davies, 2010). Chlorhexidine is a positively charged molecule that binds to negatively charged molecules in cell walls in order to enter. It is deactivated by organic matter and is more effective at alkaline pH. Its benefits over alcohol-based agents are that it does not contribute to patient hypothermia, and it is not flammable so can be used with electrocautery. It is thus the most commonly used agent for surgical site preparation.

The aim of this study was to determine the efficiency of skin preparation methodology in removing bacteria from patients pre-surgery, and to investigate how effective the bactericidal action of CHX is.

Methods

Sample collection

Ethical approval for this study was acquired from the University of Bristol's Research Ethics Committee. The subjects were 25 client-owned dogs that were to undergo abdominal surgery. No selection limitations or exclusion factors were placed on age, breed, or sex. Patients had hair clipped from the thoracic outlet to the pubic symphysis, and to the lateral skin edges.

A nylon-flocked swab (eSwab™, Copan Diagnostics, USA) was used to sample the abdominal midline skin after clipping. The swab was rubbed for five seconds then replaced in transport medium. To prepare the skin for surgery, non-woven swabs (Sofsorb™, Synergy Health, UK) were soaked in a 50:50 v/v solution of Vetasept® chlorhexidine (Animalcare, UK), giving a concentration of 2% CHX in the in-use solution. Skin scrub methods were changed between patients, alternating between the linear method and concentric circle method. The skin scrub was ceased after 5 minutes if a dry swab appeared visibly clean after rubbing across the midline and each outer edge of the clipped site, or was continued until such time that a swab did appear clean. A "post-scrub" flora sample was taken using the method previously described. All skin preparation and sampling was performed by one person to prevent variability.

Bacterial culture and CHX resistance testing

One hundred microliters of the transport medium from each swab was cultured on MacConkey agar with salt (MAC; selective for Enterobacteriaceae) and mannitol salt agar (MSA; selective for staphylococci) (Thermo Fisher Scientific, UK). The plates were incubated for 24 hours at 37°C, after which the number of colonies was counted. Colonies from post-preparation swabs were then subcultured onto blood agar (Thermo Fisher) for further identification of bacteria. Bacteria were then identified by standard biochemical testing.

Bacteria that were present on the skin following the use of CHX were investigated for CHX resistance using a broth dilution method to determine the minimum inhibitory concentration. Bacteria were cultured in brain heart infusion broth overnight at 37°C and then tested for the ability to grow in this broth containing dilutions of CHX between 0.04% and 3.125% v/v using an adaptation of the CLSI broth microdilution methodology (http://clsi.org/).

Statistical analysis

Graphpad Prism 5.0 (GraphPad Software, USA) was used for statistical analysis. A paired Wilcoxon signed rank test was used to test reductions in bacterial numbers before and after skin preparation in individual animals, a Mann–Whitney U test to look at differences in numbers between skin preparation techniques, and a one-tailed Fisher's exact test to determine significance of differences in the frequency of animals having any bacteria present after skin preparation. Significance was defined as having a P value less than 0.05.

Results

Bacteria were grown on MAC agar for the enumeration of Enterobacteriaceae and MSA for staphylococci. It was noted that *Bacillus* spp. were frequently cultured on both media, and these bacteria were included in total counts. Both the linear (**Figure 1**) and concentric circle (**Figure 2**) preparation methods

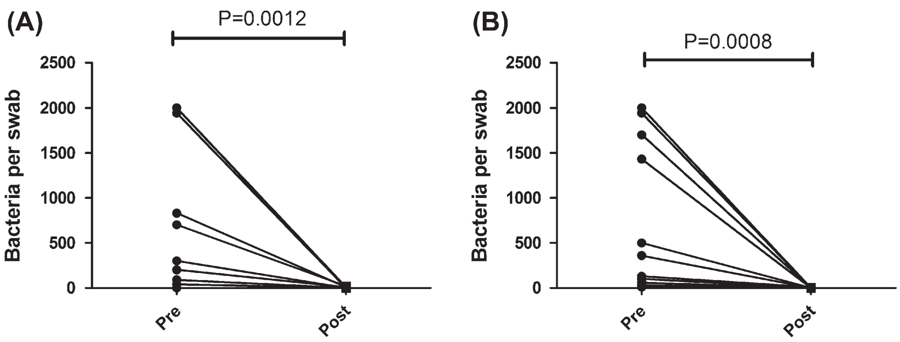

Figure 1. The decrease in the number of bacteria between pre-preparation and post-preparation using the linear method for bacteria grown on MAC plates (A) and MSA plates (B). N = 13 swabs

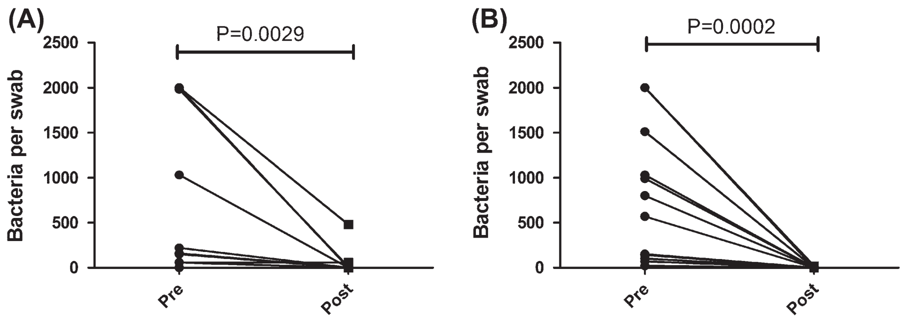

Figure 2. The decrease in the number of bacteria between pre-preparation and post-preparation using the concentric circle method for bacteria grown on MAC plates (A) and MSA plates (B). N = 12 swabs

Table 1. Bacterial species isolated after skin preparation and their resistance to chlorhexidine.

Sample	Species	Chlorhexidine MIC (%)
4 MAC	*E. coli*	<0.04
9 MAC	*Bacillus*	<0.04
9 MSA	*Bacillus*	0.18
11 MAC	*Shigella*	3.125
15 MAC	*Bacillus*	3.125
20 MAC	*Klebsiella*	<0.04
20 MSA	*Klebsiella*	<0.04
23 MAC	*Bacillus*	<0.04
24 MAC	*Bacillus*	0.75
25 MAC	*Bacillus*	<0.04

significantly decreased the number of bacteria present. This decrease was a mean of 99% on bacteria grown on MAC and MSA for the linear method and average decreases of 89% (MAC) and 99.9% (MSA) for the concentric circle method. These differences were not significant.

Of the 25 dogs sampled, eight (32%) had bacteria present after preparation, and these represented 10 species, as two dogs had two species of bacteria remaining after preparation (**Table 1**). Three of these dogs were prepared using the concentric circle method and five using the linear method. There was no statistically significant difference between groups in terms of the likelihood of bacteria remaining after preparation. The bacteria isolated after skin preparation were identified as *E. coli*, *Bacillus* spp., *Klebsiella* spp. and *Shigella* spp. Four of the 10 were resistant to CHX at one of the dilutions tested, and two were resistant at minimum inhibitory concentrations (MICs) of 3.125%, the maximum concentration tested. These were one *Shigella* and one *Bacillus*, isolated from different dogs.

Discussion

Pre-surgical skin preparation

Anecdotal evidence states that the concentric circle method prevents recontamination while the linear method provides more friction. This additional friction did not lend any advantage, with results showing no significant difference between the methods. These suggest that the technique used may be that of personal preference, and there is no observable benefit to the selection of either method. It should be noted that even after preparation some samples from patients prepared using each method had residual bacteria present. This indicates that the mechanical scrubbing action of swabbing alone is not sufficient to remove bacteria. It is for this reason that CHX is used as a disinfectant, so that chemical killing can complement mechanical removal.

CHX resistance

In the majority of cases, SSIs are caused by commensal rather than extraneous flora (Reichman & Greenberg, 2009). Previous studies have found chlorhexidine resistance in *Proteus* spp. (Dance, Pearson, Seal, & Lowes, 1987), *Klebsiella* spp. (Evans, Knowles, Werrett, & Holt, 2009) and *Pseudomonas* spp. (Levy 002). The existence of CHX resistance in commensal flora is significant within the veterinary profession as 77% of practices use CHX (Evans et al., 2009). SSIs create an increased economic burden for practice and owner, with increased visits, additional therapies and surgeries, diagnostic procedures and lengthy hospitalisation often being necessary (Nicoll, Singh, & Weese, 2014). The welfare of the patient is also at risk as additional stress and can lead to increased morbidity. Surgical procedures and anaesthesia always carry risks, especially in compromised patients, and inflammation leads to pain and poor welfare. The patient may need additional analgesia, which is a further cost and can carry risks.

Chlorhexidine works by first damaging the cell wall in order to gain access to and adhere to the plasma membrane, causing lysis (Maillard, 2002). The presence of CHX resistance could be due to various factors. *Bacillus* spp. are able to produce endospores, which are able to survive many stresses, including disinfection. *Shigella* spp. have a different mechanism of resistance. One such mechanism involves the presence of the *mar* phenotype (Cohen, Hachler, & Levy, 1993), which causes resistance to both antibiotics and disinfectants and is associated with the production of bacterial efflux pumps. Upregulation of this can be caused by external stimuli, such as the use of biocides (Russell, Tattawasart, Maillard, & Furr, 1998), so it is possible that biocide use at sublethal concentrations can select for resistant bacteria, just as underdosing of antibiotics can select for antibiotic resistance.

This study shows the importance of monitoring bacterial resistance to biocides in the clinical setting, so that appropriate agents may be used for killing bacteria, and it is possible that biocides should be rotated so that resistance cannot build up in bacteria. Alternative biocides are available. Iodine povacrylex has been found by some studies to be more efficacious than CHX (Hemani & Lepor, 2009; Swenson et al., 2009). Another biocide with limited research is parachoroxylenol, which has been shown to be efficacious (Zinn, Jenkins, Swofford, Harrelson, & McCarter, 2010) and safe for mucous membranes and ears, unlike CHX which can be an irritant. This, however, is not a well-studied area of veterinary or human medicine, but is key to proper infection control. Although the sample size in this study was small, the significant results show a need for further investigation of biocide resistance in bacteria.

Acknowledgements

The authors would like to thank the surgeons, nurses and animal care assistants of the Langford Small Animal Referral Hospital and Langford Small Animal Practice for their help and support during this project.

Funding

This project had no funding external to the University of Bristol.

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