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Duration of at Home Preoperative Skin Antiseptics against Common Shoulder Pathogens

Ethan Sawyer[1*], Evan Johnson[2], Lester Hubble[2],Caleb Freeman[2], Paul Watson[2]

[1]*Department of Orthopedic Surgery, Toledo University, USA*
[2]*Department of Orthopedic Surgery, Creighton University, USA*

ABSTRACT

Background: The aim of this study was to determine the duration of efficacy of 0.13% benzalkonium chloride (BAC), 4% chlorhexidine and 70% Ethanol in decreasing MRSA and *Cutibacterium acnes* (*C. acnes*) colonization and regrowth when plated on culture media. Specifically, the study was aimed at determining how long the antiseptics can be applied preoperatively while remaining effective.

Methods: Sterile discs saturated with either 0.13% BAC, 4% chlorhexidine, or 70% ethanol were placed on 5% sheep blood agar plates cultured with either MRSA or *C. acnes*. The clearance of bacteria surrounding the discs was measured at 24 hour increments for a total of 120 hours to determine the original zone of inhibition (ZOI) as well as any bacterial regrowth.

Result: 4% chlorhexidine and 0.13% BAC produced a consistent ZOI that increased in size and that did not permit any significant regrowth of the bacteria over 120 hours. 70% ethanol did not show any ZOI for either MRSA or *C. acnes* at any time point.

Conclusion: The results of this study demonstrate the potential of using BAC for preoperative shoulder preparation given the duration of effect. Further study is warranted to determine the *in vivo* effectiveness of this formulation of BAC.

Keywords: Antiseptic; Pathogens; *C. acnes*; *S. aureus*; Sterile discs

INTRODUCTION

Prevention and control of surgical site infections is imperative for the health and safety of patients. Infection after shoulder surgery often results in functional deficit and patient dissatisfaction [1,2,3]. When surgical site infections do occur, many are believed to be a result of surgical site contamination by a patient's own microbiota. Common constituents of patients' dermal microbiota include staphylococcal species and *Cutibacterium acnes* especially on the shoulder [4,5]. The use of perioperative antiseptic agents has been shown to reduce post-operative infection rates [6]. Both chlorhexidine and benzalkonium chloride (BAC) have been shown to be effective at home pre-surgical whole body antiseptics in preventing surgical site infection [5,7,8]. Although these antiseptics are proven to be effective in preventing surgical site infection, the duration of effectiveness of these antiseptics before surgery has not been determined. One potential hypothesis for these infections is that the efficacy

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Corresponding author Ethan Sawyer, Department of Orthopedic Surgery, Toledo University, USA, E-mail: ethansawyer@gmx.us

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of current standard antiseptics wanes over time allowing for regrowth of patients' own microbiota.

The aim of this study was to determine the duration of efficacy of 0.13% BAC, 4% chlorhexidine and 70% Ethanol in decreasing Methicillin Resistant Staphylococcus aureus (MRSA) and *Cutibacterium acnes* (*C. acnes*) colonization and regrowth over an extended time period when plated on culture media. Specifically, we wanted to determine how long they can be applied before surgery and still remain effective.

METHODS

A KWIKSTIK™ *Cutibacterium acnes* ATCC 11827 system was utilized to establish a broth reservoir of *C. acnes* bacteria. An aliquot of the *C. acnes* broth was streaked on a 5% sheep blood agar using a calibrated inoculating loop and incubated under anaerobic conditions for 48 hours at 37˚C. A calibrated inoculating loop was utilized to culture an existing MRSA agar plate and inoculate a 5% sheep blood agar plate. The plate was incubated under aerobic conditions for 48 hours at 37˚C.

A 0.5 McFarland standard solution was created for both the MRSA and *C. acnes* bacteria strains, by using a calibrated inoculating loop to transfer bacteria from the incubated blood agar plates to a vial of saline with 0% absorbance until the absorbance of the vial solution was between 0.08 and 0.1%.

A bacteria lawn was created by using a cotton applicator to evenly distribute an aliquot of the 0.5 McFarland standard MRSA solution across the surface of a 5% sheep blood agar plate. This process was repeated using the 0.5 Mcfarland standard MRSA solution and the 0.5 McFarland standard *C. acnes* solution until eight bacteria lawns of each solution were created.

50 ml of 4% chlorhexidine gluconate (Hibiclens™, Mölnlycke Health Care., Norcross, Georgia) solution was pipetted onto a 1 cm diameter Sigma Aldrich Sterile Disk in a sterile petri dish and left for 45 minutes to dry. This process was repeated using a 70% Ethanol solution (Sigma-Aldrich Inc., St. Louis, Missouri), and BAC 0.13% (Steriolotion™, Germcure Inc., Houma, Louisiana) ultimately creating three populations of antiseptic coated disks. Flame sterilized forceps were used to transfer one coated disk of each antiseptic onto each inoculated blood agar plate.

Both the MRSA and *C. acnes* inoculated plates were incubated at 37˚C overnight. The *C. acnes* plate incubated within an airtight anaerobic chamber containing two GasPak™ EZ Anaerobe Sachets with indicators to create an anaerobic condition preferential for *C. acnes* growth. GasPak™ EZ Anaerobe Sachets were replaced any time the anaerobic chamber was opened. The MRSA were grown in aerobic conditions.

The plates were removed from the incubator every 24 hours for a growth period of 120 hours to take photographs and qualitative measurements of the zone of inhibition (ZOI) surrounding each antiseptic coated disc. Measurements were performed for a total of 120 hours for the MRSA plates and a total of 144 hours for the *C. acnes* plate due to a lack of bacterial lawn growth at the 24-hour interval.

Three methodologies were utilized to perform quantitative measurements of the ZOI surrounding each antiseptic coated disk. The first methodology was to perform manual measurements of the ZOI and the sterile disc using a metric ruler. The second methodology was to use the free internet software program, ImageJ, to perform digital measurements of the ZOI. ImageJ utilizes the pixels of the digital photographs taken and the known standard diameters of the agar plates and sterile discs to quantitatively measure the ZOI. Similarly, an additional software program, Preview, was used to analyze the pixels of the digital photographs and known measurements of the disc and agar plate to derive metric measurements of the ZOI. The results were recorded with both manual and digital measurements using multiple software programs. Two different researchers made the manual measurements independently and two different researchers made the digital measurements independently.

For increased validity of the measurement trends, the zones of inhibition were repeated by another researcher using the digital measurements capabilities of Apple's Preview program and by a third researcher by manually measuring the zones on the computer and calculating the actual values using ratios related to the 1 cm sterile discs.

RESULTS

The 70% alcohol did not show any ZOI for either MRSA or *C. acnes* at any time point throughout the study. Both chlorhexidine and BAC produced consistent zones of bacterial inhibition that did not permit any significant regrowth of the bacteria over the course of 120 hours (**Figure 1**). These results are shown in **Table 1**. From day 1 to 5, Chlorhexidine produced a clear ZOI with a 10% increase in size for MRSA and a 4% increase for *C. acnes*. BAC also produced a clear ZOI with a size increase of 4% against MRSA and 12% against *C. acnes* (**Table 2**). The values after 24 hours of *C. acnes* growth plate were not able to be recorded on the initial manual measurements because the contrast between the ZOI and surrounding bacteria was too slight to obtain a measurement (**Figure 2**).

Table 1: Chlorhexidine inhibition of MRSA.

Chlorhexidine vs. MRSA	Manual-1	Manual-2	Digital-1	Digital-2
Day 1	1.97 cm	2.16 cm	2.19 cm	2.29 cm
Day 2	2.33 cm	2.17 cm	2.28 cm	2.28 cm
Day 3	2.33 cm	2.17 cm	2.25 cm	2.32 cm
Day 4	2.33 cm	2.17 cm	2.20 cm	2.3 cm
Day 5	2.33 cm	2.16 cm	2.20 cm	2.24 cm
Final-Initial Measured ZOI	0.36 cm	0.00 cm	0.01 cm	-0.05 cm

Note: Each value represents the mean of 8 individual trials

Table 2: Chlorhexidine inhibition of *C. acnes*.

Chlorhexidine vs. *C. acnes*	Manual-1	Manual-2	Digital-1	Digital-2
Day 1	Light Growth	2.91 cm	3.00 cm	2.90 cm
Day 2	3.54 cm	3.25 cm	3.34 cm	3.11 cm
Day 3	3.39 cm	3.31 cm	3.35 cm	3.28 cm
Day 4	3.45 cm	3.32 cm	3.33 cm	3.25 cm
Day 5	3.59 cm	3.38 cm	3.30 cm	3.27 cm
Final-Initial Measured ZOI	0.05 cm	0.47 cm	0.30 cm	0.37 cm

Note: Each value represents the mean of 8 individual trials

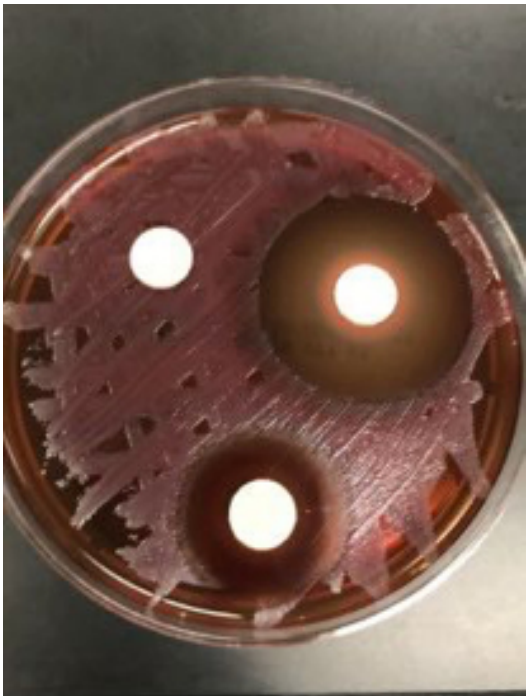

Figure 1: *C. acnes* inhibition at 120 hours by ethyl alcohol, chlorhexidine and BAC.

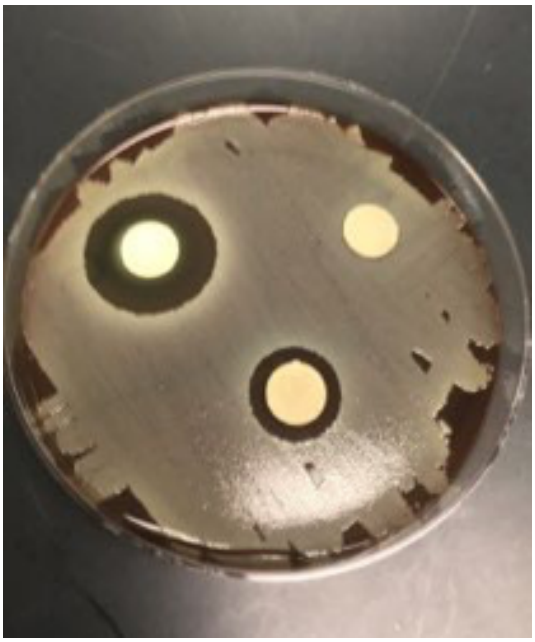

Figure 2: MRSA inhibition at 120 hours by ethyl alcohol, chlorhexidine and BAC.

A small hazy ring that appeared around the outer edges of the zones of inhibition on the BAC vs. *C. acnes* trials after 48 hours that did not appear to change in quality or size over the course of 120 hours and did not appear to display any signs of bacterial regrowth (**Table 3**). There also appeared to be an increased buildup of bacteria surrounding the chlorhexidine ZOI vs. MRSA trials as observed by the white ring of increased intensity surrounding the ZOI on these plates. This suggests there may be increased MRSA growth in the areas immediately adjacent to the ZOI (**Table 4**).

Table 3: BAC vs. MRSA.

BAC vs. MRSA	Manual-1	Manual-2	Digital-1	Digital-2
Day 1	1.65 cm	1.52 cm	1.64 cm	1.65 cm
Day 2	1.65 cm	1.52 cm	1.64 cm	1.62 cm
Day 3	1.66 cm	1.52 cm	1.63 cm	1.64 cm
Day 4	1.66 cm	1.56 cm	1.67 cm	1.63 cm
Day 5	1.66 cm	1.59 cm	1.74 cm	1.72 cm
Final-Initial Measured ZOI	0.01 cm	0.07 cm	0.10 cm	0.07 cm

Note: Each value represents the mean of 8 individual trials

Table 4: BAC vs. *C. acnes*.

BAC vs. *C. acnes*	Manual-1	Manual-2	Digital-1	Digital-2
Day 1	Light Growth	2.19 cm	2.37 cm	2.23 cm
Day 2	2.66 cm	2.37 cm	2.65 cm	2.35 cm
Day 3	2.61 cm	2.46 cm	2.70 cm	2.56 cm
Day 4	2.60 cm	2.54 cm	2.67 cm	2.61 cm
Day 5	2.61 cm	2.57 cm	2.69 cm	2.64 cm
Final-Initial Measured ZOI	-0.05 cm	0.38 cm	0.32 cm	0.41 cm

Note: Each value represents the mean of 8 individual trials

DISCUSSION

Even after the many recent advances in surgical technique, postoperative joint infection remains a common complication for patients. Unfortunately, many of these infections are a result of surgical contamination by a patient's own microbiota. *S. aureus* and *C. acnes* frequently colonize the skin and are pathogens that frequently cause postoperative shoulder infections [9-11]. These infections result in increased morbidity and mortality, and ultimately higher health care costs for both the patient and the health care system [12].

At home preoperative cleaning protocols have been used to reduce pathogens on the skin but there is some debate about the efficacy of chlorhexidine showering at lowering surgical site infections, and a recent meta-analysis of the available clinical trials suggests no appreciable benefit for preoperative whole-

body chlorhexidine showering in the prevention of surgical site infections [13]. This study demonstrates the sustained antimicrobial activity of both chlorhexidine and BAC against the micro-organisms *S. aureus* and *C. acnes in vitro* for over 120 hours. The 0.13% BAC formulation used here exhibited similar effectiveness and duration as 4% chlorhexidine. The two agents created clear, sustained zones of inhibition with no bacterial regrowth over a period of 5 days. These results are particularly striking when contrasted to the efficacy of the commonly used antiseptic 70% ETOH, which failed to prevent bacterial regrowth for either organism. Although chlorhexidine did create a larger initial ZOI, there was a slight decrease in the area over time from the 48 hour to 120 time periods while the BAC had a slight increase in the ZOI. The fact that both chlorhexidine and BAC demonstrated minimal regrowth demonstrates their potential for at home skin decontamination of the surgical site. This data is significant in that prior studies have shown that chlorhexidine is ineffective at reducing *C. acnes* colonization prior to shoulder surgery [14], suggesting that this formulation of BAC could be a possible alternative agent. It is also important to note that there was a white zone of overgrowth around the ZOI on plates treated with chlorhexidine that was not visible on BAC-treated plates. This suggests a possible risk for overgrowth of MRSA in areas missed during at home preoperative cleaning with chlorhexidine; this overgrowth was not seen on plates treated with BAC. As well this formulation of BAC is a leave on antiseptic as tested in this study whereas chlorhexidine is usually used as a home wash in the shower and washed off after application. This could account for its lack of effectiveness in certain studies.

Benzalkonium chloride is commonly used in health care as a bactericidal agent in surface disinfectants, nasal sprays and eye drops with minimal skin irritation. This study demonstrates its potential as a preoperative skin cleaning agent prior to surgery with sustained efficacy against *C. acnes*. To our knowledge, the efficacy of BAC against *S. aureus* and *C. acnes* in comparison to chlorhexidine and 70% ETOH has not been previously investigated. As such, this is the first study to demonstrate a similar ability of BAC to chlorhexidine in preventing *in vitro* bacterial MRSA and *C. acnes* regrowth following antiseptic treatment. Further work is necessary to determine whether BAC exhibits similar effectiveness *in vivo*.

Currently Benzoyl Peroxide is being used against *C. acnes* as an additional antiseptic for preoperative shoulder surgery preparation [15]. It has been shown to have rapid effect, making a two day preparation sufficient for reducing bacteria on the skin [16,17]. The results of this study suggest that BAC may be another alternative with equally effective preparation times.

This study has several important limitations. First, these agents were tested against microbial cultures of *S. aureus* and *C. acnes*. The *in vitro* nature of this study does not account for numerous variables relating to interactions with the skin that would be seen under *in vivo* conditions. These interactions include daily bathing and physical exfoliation of the skin during showers or other daily activities, uptake and degradation of the applied antiseptic by host epithelial cells, and day to day environmental changes of the surgical area caused by changes in clothing, sweating, etc. As such, it remains to be seen whether the antiseptics will demonstrate a similar efficacy against these pathogens when used as preoperative surgical preparation *in vivo*. Second, although BAC in this study demonstrated similar impairment of bacterial regrowth to chlorhexidine, these results may be variable depending on the strain of bacteria isolated, as modes of resistance vary between strains. Third, although 0.10%-0.13% BAC is the concentration suggested by the United States of America Food and Drug Administration for skin sanitizers and first aid antiseptics [18], this study did not attempt to evaluate different concentrations to determine the minimal levels needed for inhibition or any risks or toxicity towards normal skin, thus further investigation is necessary to identify optimal concentrations for *in vivo* studies.

CONCLUSION

In conclusion, both BAC and chlorhexidine were effective in preventing *in vitro* regrowth of both *S. aureus* and *C. acnes* over 5 days with a single application. Both agents were superior to 70% ETOH which showed no inhibition at all time points. These pathogens are known to be found in the normal flora of skin and are common causes of shoulder post-operative joint infection. This study demonstrates the potential of using BAC for preoperative shoulder preparation; however, further study is warranted to determine the *in vivo* effectiveness of this formulation of BAC.

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CONFLICT OF INTEREST

Paul Watson, MD and/or an immediate family member own stock in two related infection control companies: Steiros LLC and bioblockade LLC.

REFERENCES

1. Athwal GS, Sperling JW, Rispoli DM, Cofield RH (2007) Deep infection after rotator cuff repair. J Shoulder Elb Surg 16(3): 306–11.
2. Settecerri JJ, Pitner MA, Rock MG, Hanssen AD, Cofield RH (1999) Infection after rotator cuff repair. J Shoulder Elb Surg 278(1): 1–5.
3. Strickland JP, Sperling JW, Cofield RH (2008) The results of two-stage re-implantation for infected shoulder replacement. J Bone Joint Surg Br 90(4): 460-65.
4. Brislin KJ, Field LD, Savoie FH (2007) Complications after arthroscopic rotator cuff repair. Arthrosc J Arthrosc Relat Surg 23(2): 124–8.

5. Murray MR, Saltzman MD, Gryzlo SM, Terry MA, Woodward CC, et al. (2011) Efficacy of preoperative home use of 2% chlorhexidine gluconate cloth before shoulder surgery. J Shoulder Elbow Surg 20(6): 928-933.

6. Randelli P, Castagna A, Cabitza F, Cabitza P, Arrigoni P, et al. (2010) Infectious and thromboembolic complications of arthroscopic shoulder surgery. J Shoulder Elb Surg 19(1): 97-101.

7. Everett BR, Sitton JT, Wilson M (2017) Efficacy and cost-benefit analysis of a global environmental cleaning algorithm on hospital-acquired infection rates. J Patient Saf 13(4): 207–10.

8. Watson PA, Watson L, Torress-Cook A (2012) Institution of the Steiros Algorithm(®) Outpatient Surgical Protocol reduced orthopedic surgical site infections (SSI) rates. Iowa Orthop J (2018) 32: 116–9.

9. Patel A, Calfee RP, Plante M, Fischer SA, Green A (2009) *Propionibacterium acnes* colonization of the human shoulder. J Shoulder Elb Surg 18(6): 897-902

10. Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, et al. (2007) Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med 357(7): 654–63.

11. Zeller V, Ghorbani A, Strady C, Leonard P, Mamoudy P (2007) Desplaces N.*Propionibacterium acnes*: An agent of prosthetic joint infection and colonization. J Infect 55(2): 119–24.

12. Del Pozo JL, Patel R (2009) Infection associated with prosthetic joints N Engl J Med 361(8): 787–94.

13. Chlebicki MP, Safdar N, O'Horo JC, Maki DG (2013) Preoperative chlorhexidine shower or bath for prevention of surgical site infection: A meta-analysis. Am J Infect Control 167–73.

14. Lean M, Simon B, Phadnis J, Deep J, Ling CM, et al. (2018) Application of dermal chlorhexidine antisepsis is ineffective at reducing *Proprionibacterium acnes* colonization in shoulder surgery. J Shoulder Elbow Surg 11(2): 111-115.

15. Scheer V, Bergman M, Lerm M, Serrander L, Kalén A (2019) Topical benzoyl peroxide application on the shoulder reduces *Propionibacterium acnes*: A randomized study. J Shoulder Elbow Surg 27(6): 957-961.

16. Chuah SY, Attia ABE, Long V, Ho CJH, Malempati P, et al. (2017) Structural and functional 3D mapping of skin tumours with non-invasive multispectral optoacoustic tomography. Ski Res Technol 23(2): 221-226.

17. Kosmadaki M, Katsambas A (2017) Topical treatments for acne. Clin Dermatol 35(2): 173–8.

18. Safety and effectiveness of consumer antiseptic rubs; topical antimicrobial drug acts for over-the-counter human use. Federal register. 21 cfr 310.