INFECTION

Bactericidal efficacy of hydrogen peroxide on *Cutibacterium acnes*

**Objectives**
The purpose of this study was to examine the bactericidal efficacy of hydrogen peroxide (H$_2$O$_2$) on *Cutibacterium acnes* (*C. acnes*). We hypothesize that H$_2$O$_2$ reduces the bacterial burden of *C. acnes*.

**Methods**
The effect of H$_2$O$_2$ was assessed by testing bactericidal effect, time course analysis, growth inhibition, and minimum bactericidal concentration. To assess the bactericidal effect, bacteria were treated for 30 minutes with 0%, 1%, 3%, 4%, 6%, 8%, or 10% H$_2$O$_2$ in saline or water and compared with 3% topical H$_2$O$_2$ solution. For time course analysis, bacteria were treated with water or saline (controls), 3% H$_2$O$_2$ in water, 3% H$_2$O$_2$ in saline, or 3% topical solution for 5, 10, 15, 20, and 30 minutes. Results were analyzed with a two-way analysis of variance (ANOVA) (p < 0.05).

**Results**
Minimum inhibitory concentration of H$_2$O$_2$ after 30 minutes is 1% for H$_2$O$_2$ prepared in saline and water. The 3% topical solution was as effective when compared with the 1% H$_2$O$_2$ prepared in saline or water. The controls of both saline and water showed no reduction of bacteria. After five minutes of exposure, all mixtures of H$_2$O$_2$ reduced the percentage of live bacteria, with the topical solution being most effective (p < 0.0001). Maximum growth inhibition was achieved with topical 3% H$_2$O$_2$.

**Conclusion**
The inexpensive and commercially available topical solution of 3% H$_2$O$_2$ demonstrated superior bactericidal effect as observed in the minimum bactericidal inhibitory concentration, time course, and colony-forming unit (CFU) inhibition assays. These results support the use of topical 3% H$_2$O$_2$ for five minutes before surgical skin preparation prior to shoulder surgery to achieve eradication of *C. acnes* for the skin.

Cite this article: Bone Joint Res 2019;8:3–10.

**Keywords:** *Cutibacterium acnes*, Shoulder, Hydrogen peroxide, Infection, Treatment, Shoulder arthroplasty

**Article focus**
- The purpose of this study is to investigate the antibacterial potential of H$_2$O$_2$ for the eradication of *C. acnes*.

**Key messages**
- Hydrogen peroxide has both bactericidal as well as bacteriostatic properties against *C. acnes*. The minimum time for hydrogen peroxide to produce its bactericidal effect on *C. acnes* is five minutes.

**Strengths and limitations**
- This is the first investigation to examine the bactericidal properties of hydrogen peroxide for *C. acnes*.
- This is a proof-of-concept pilot study and therefore only utilizes lab isolates of *C. acnes*; it is unknown if hydrogen peroxide is equally as effective with clinical isolates.
- The tissue toxicity of hydrogen peroxide at the minimal inhibitory concentration found in this investigation is unknown.

**Introduction**
*Cutibacterium acnes* (*C. acnes*), formerly named *Propionibacterium acnes*, is one of the most common causative organisms causing infection following shoulder surgery. Multiple attempts have been made to provide consistent, reproducible methods of...
decolonization and/or eradication of this troublesome organism preoperatively in an effort to reduce the risk of surgical site contamination and infection.\textsuperscript{3-10} \textit{Cutibacterium acnes} is a slow-growing, facultative anaerobic Gram-positive bacillus commonly residing in the deep dermal layer of the skin within the pilosebaceous glands and hair follicles.\textsuperscript{4,5,9,11,12} This location not only makes \textit{C. acnes} difficult to eradicate during surgical skin preparation because of poor penetration, but also puts the patient at risk of contamination of the shoulder joint due to repeated contact with this layer beneath the epidermis.\textsuperscript{2,4-6,10,11,13,14} It has been hypothesized that \textit{C. acnes} inoculates the surgical wound once incision is made through the pilosebaceous glands.\textsuperscript{11,15}

Additional attempts have been made to decolonize the skin utilizing antibiotics, either systemically or via topical application, with varying degrees of success.\textsuperscript{5,8,16-18} This bacteria has been shown to be susceptible to several antibiotics including penicillin G, amoxicillin, cephalothin, ceftriaxone, clindamycin, doxycycline, and rifampin.\textsuperscript{16,17} The issue that arises following administration of antibiotics is the emergence of resistance, with several recent reports of increasing resistance to clindamycin, doxycycline, tetracycline, minocycline, and erythromycin.\textsuperscript{17,19-22} Despite this susceptibility, recent studies\textsuperscript{4,15} have demonstrated prophylactic intravenous antibiotics prior to skin incision to be ineffective and to continue to result in positive \textit{C. acnes} cultures. Thus far, routine skin preparation and intravenous perioperative antibiotics have not been shown to provide antimicrobial protection against \textit{C. acnes}.

Two recent studies\textsuperscript{5,9} evaluated the effectiveness of topically applied benzoyl peroxide perioperatively. These studies demonstrated an almost 50\% reduction in positive superficial cultures\textsuperscript{5,9} and, when combined with topical clindamycin, positive deep cultures decreased from 19.6\% to 3.1\%.\textsuperscript{3} Topical benzoyl peroxide has been proven to be an effective treatment, as it is lipophilic and can penetrate the deep dermal layer as well as release free oxygen radicals.\textsuperscript{23-26} These free radicals result in oxidation of proteins in bacterial cell membranes with subsequent cell death. While this method seems to be effective, it utilizes an antibiotic and can develop resistance to treatment. Furthermore, these treatments require patient compliance with the perioperative topical treatment regimen. Allhorn et al\textsuperscript{27} describe a novel antioxidant enzyme, radical oxygenase of \textit{Propionibacterium acnes} (RoxP), which is produced by the bacteria to protect against oxidation and is hypothesized to facilitate its survival on skin. We are unaware of any studies describing the effectiveness of this enzyme when the bacteria have not had time to adapt to exposure to a new strongly oxidative product.

Hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) is cheap, widely available, and can be applied at the time of surgery. Given that H\textsubscript{2}O\textsubscript{2} is not an antibiotic, there is no risk for development of resistant \textit{C. acnes} strains. In theory, the aerobic environment created in the presence of H\textsubscript{2}O\textsubscript{2} should be detrimental to anaerobic bacteria. It has been well established that polymorphonuclear leukocytes (PMNs) eliminate microorganisms through the generation of reactive oxygen free radicals, and utilize H\textsubscript{2}O\textsubscript{2} to facilitate this respiratory burst mechanism resulting in cell death.\textsuperscript{28}

The purpose of this study is to investigate the antibacterial potential of H\textsubscript{2}O\textsubscript{2} for the eradication of \textit{C. acnes}. To our knowledge, no study has been performed evaluating the efficacy of H\textsubscript{2}O\textsubscript{2} in reducing the burden of \textit{C. acnes}. We hypothesize that H\textsubscript{2}O\textsubscript{2} will be an effective bactericidal treatment against \textit{C. acnes}.

**Materials and Methods**

\textit{Cutibacterium acnes} (ATCC 6919) was cultured in tryptic soy broth (TSB; Becton, Dickinson and Company, Franklin Lakes, New Jersey) with 5\% defibrinated sheep blood (DSB) and plated on TSB agar with 5\% DSB (TSB-DSB agar), at 37\°C under anaerobic conditions using Gas-pak (Becton, Dickinson and Company). Both liquid media and agar plates were pre-reduced for 24 hours before \textit{C. acnes} culture. All pre-inoculums were made from a single colony in 2 ml broth.

Two inoculums of 25 ml each were started with a 1:100 dilution from 24-hour pre-inoculums (10\° colony-forming units (CFUs)). The cell number was calculated after a colony count of serial dilutions. Serial dilutions were prepared from 100 ml samples taken at 0, 6, 24, 30, 48, 54, 78, and 97 hours of growth, and cultured in duplicate on TSB-DSB agar. The plates were incubated at 37\°C for five days under anaerobic conditions. After the incubation period, viable colonies were counted and the number of CFUs per millilitre of culture (CFU/ml) was determined. The generation time (gt) was calculated according to Hall et al,\textsuperscript{29} using the following formula, where \textit{CFU\textsubscript{fi}} and \textit{CFU\textsubscript{fi}} are final and initial CFU per ml, \textit{t} and \textit{t} are final and initial times in hours, and \textit{K} = log\textsubscript{2} = 0.301:

\[
gt = \frac{K(t_f - t_i)}{\log(CFU_{fi}) - \log(CFU_{fi})}
\]

Diolusions of 1\%, 3\%, 4\%, 6\%, 8\%, and 10\% H\textsubscript{2}O\textsubscript{2} were freshly prepared with Milli-Q water or 0.85\% sodium chloride (NaCl) (saline) from a 30\% H\textsubscript{2}O\textsubscript{2} solution (Fisher Bioreagents, Pittsburgh, Pennsylvania) and compared with a commercial solution of stabilized 3\% topical H\textsubscript{2}O\textsubscript{2} in water (topical solution) (Henry Schein Inc., Melville, New York). Pre-inoculums and 1:100 dilution inoculums were grown in TSB-DSB broth. At 50 hours of culture (10\° CFUs), bacteria were centrifuged at 4300 \times g for ten minutes, resuspended in saline and divided into 15 tubes. Each tube was treated for 30 minutes at room temperature.
Bactericidal efficacy of hydrogen peroxide on *Cutibacterium acnes*

Table 1. Results of a power analysis of the five-minute data from the time course experiment

| Power for analysis of variance (ANOVA) |
|----------------------------------------|
| **Data source: 5 mins 3% H₂O₂ in saline vs 3% topical H₂O₂** |
| Power | 1 |
| Difference in means | 65.73 |
| Standard deviation | 13.52 |
| Groups, n | 3 |
| Group size, n | 5 |
| Alpha | 0.05 |
| **Data source: 5 mins 3% H₂O₂ in water vs 3% topical H₂O₂** |
| Power | 1 |
| Difference in means | 74.13 |
| Standard deviation | 13.52 |
| Groups, n | 3 |
| Group size, n | 5 |
| Alpha | 0.05 |

Growth curve of purified strain of *C. acnes*. Bacteria were grown in tryptic soy broth–defibrinated sheep blood (TSB-DSB) at 37°C under anaerobic conditions. Samples were taken at 0, 6, 24, 30, 48, 54, 78, and 97 hours of growth and culture aliquots were plated on TSB-DSB agar. The calculated generation time (gt) is 4.39 hours. Data are presented as the mean (standard deviation) of two measurements. CFUs, colony-forming units.

Fresh H₂O₂ dilutions in water and saline were prepared as previously mentioned for dose response. For this experiment, pre-inoculums and 1:50 dilution inoculums were grown in TSB without blood. Bacteria were centrifuged at 4300 × g for ten minutes, washed in saline once, and divided into 19 tubes. Each one was incubated in one of the following conditions: 3% H₂O₂ prepared in saline; 3% H₂O₂ prepared in water; or 3% topical solution for 0, 5, 10, or 15 minutes at room temperature. Saline-only and water-only controls were incubated for 15 minutes. Bacteria were centrifuged at 10 000 × g for five minutes, which resulted in final incubation times of 5, 10, 15, and 20 minutes. Cells were resuspended in saline and stained with Live/Dead BacLight bacterial viability kit (Molecular Probes, Invitrogen, Waltham, Massachusetts) following the manufacturer’s instructions. A standard curve for each experiment was created by mixing the percentages of live and dead bacteria in saline. To obtain dead bacteria, tubes were exposed to 95°C for ten minutes. Plates were exposed to 480 nm for excitation with 520 nm (green) and 620 nm (red) emission.

Statistical analysis. A two-way analysis of variance (ANOVA) with Tukey’s test for multiple comparisons of time course data was performed with GraphPad Prism version 7 (GraphPad Software, La Jolla, California). Differences were considered significant when the p-value was less than 0.05. A one-way ANOVA with Dunnett’s post hoc test was performed to compare timepoints on each 3% H₂O₂ solution. A power analysis of time course data was performed with SigmaPlot 13.0 (Systat Software, Inc., San Jose, California) (Table I).

Results

Reconstituted *C. acnes* was first plated in TSB-DSB agar in anaerobic conditions to produce single colonies. Controls included bacteria grown in aerobic conditions and non-cultured plates in both anaerobic and aerobic conditions. No colonies were observed in any of the controls. All colonies of *C. acnes* in plates shared the same characteristics: circular, convex, smooth, white, and opaque. We then characterized the growth of this *C. acnes* purified strain from ATCC for up to 97 hours of culture (Fig. 1). Our
calculations for generation time (gt) were 4.39 hours, similar to the 5.1 hours previously described by Hall et al., who studied intraocular clinical isolates of subjects with chronic postoperative endophthalmitis.

For the consecutive experiments, we calculated that 50 hours of culture were necessary to obtain $10^9$ CFUs.

Bacteria were treated for 30 minutes with different percentages of H$_2$O$_2$ and compared with a commercial topical solution of stabilized 3% H$_2$O$_2$. Since the topical solution is stabilized in water, we compared a fresh diluted H$_2$O$_2$ solution in both saline and water. Plates were incubated for five days in anaerobic conditions to allow the development of colonies. Results show that all the concentrations tested for H$_2$O$_2$ have effective bactericidal properties (Fig. 2). Negative controls of saline only and water only showed normal bacterial growth (Fig. 2a).

The graph in Figure 2b shows that 1% H$_2$O$_2$ is the minimum bactericidal concentration (MBC) for C. acnes. The topical solution of H$_2$O$_2$ is as effective as 1% of a freshly prepared solution.

To corroborate the bactericidal effect of the 3% H$_2$O$_2$ solutions, treated C. acnes were seeded as lawn to observe growth inhibition (Fig. 2c). Results show that all H$_2$O$_2$ dilutions tested inhibit C. acnes growth. These results indicate that H$_2$O$_2$ has both a bactericidal and a bacteriostatic effect on this bacterium.

The minimum time required by H$_2$O$_2$ to produce its bactericidal effect on C. acnes was tested (Table II). The 3% H$_2$O$_2$ solutions as described previously were incubated for 5, 10, 15, and 20 minutes, and viability was assayed with Live/Dead BacLight bacterial viability assay (Fig. 3a). The results showed that, starting from five
Bactericidal efficacy of hydrogen peroxide on Cutibacterium acnes

The 3% topical solution significantly decreases the viability of C. acnes compared with the water-only control \((p < 0.0001)\), and it was superior in its bactericidal effect compared with 3% \(\text{H}_2\text{O}_2\) prepared either in saline or in water \((p < 0.0001)\). Bacteria treated for five minutes with each of the tested solutions were grown as lawn to further assess for growth inhibition. All treatment solutions inhibited bacterial growth except the controls with water or saline.

Table II. Results of the two-way analysis of variance (ANOVA) with Tukey’s test for multiple comparisons was performed on the time course data

| Test groups                          | Mean difference | 95% CI          | Adjusted p-value |
|--------------------------------------|-----------------|-----------------|------------------|
| **Time: 0 mins**                     |                 |                 |                  |
| 3% \(\text{H}_2\text{O}_2\) in saline vs 3% \(\text{H}_2\text{O}_2\) water | 9.418           | -23.07 to 41.9  | 0.7663           |
| 3% \(\text{H}_2\text{O}_2\) in saline vs 3% topical \(\text{H}_2\text{O}_2\) | 9.418           | -23.07 to 41.9  | 0.7663           |
| 3% \(\text{H}_2\text{O}_2\) in water vs 3% topical \(\text{H}_2\text{O}_2\) | 0               | -32.48 to 32.48 | > 0.9999         |
| **Time: 5 mins**                     |                 |                 |                  |
| 3% \(\text{H}_2\text{O}_2\) in saline vs 3% \(\text{H}_2\text{O}_2\) in water | -8.396          | -40.88 to 24.09 | 0.8091           |
| 3% \(\text{H}_2\text{O}_2\) in saline vs 3% topical \(\text{H}_2\text{O}_2\) | 65.73           | 33.25 to 98.22  | < 0.0001*        |
| 3% \(\text{H}_2\text{O}_2\) in water vs 3% topical \(\text{H}_2\text{O}_2\) | 74.13           | 41.64 to 106.6  | < 0.0001*        |
| **Time: 10 mins**                    |                 |                 |                  |
| 3% \(\text{H}_2\text{O}_2\) in saline vs 3% \(\text{H}_2\text{O}_2\) in water | -8.674          | -41.16 to 23.81 | 0.7977           |
| 3% \(\text{H}_2\text{O}_2\) in saline vs 3% topical \(\text{H}_2\text{O}_2\) | 66.75           | 34.26 to 99.23  | < 0.0001*        |
| 3% \(\text{H}_2\text{O}_2\) in water vs 3% topical \(\text{H}_2\text{O}_2\) | 75.42           | 42.94 to 107.9  | < 0.0001*        |
| **Time: 15 mins**                    |                 |                 |                  |
| 3% \(\text{H}_2\text{O}_2\) in saline vs 3% \(\text{H}_2\text{O}_2\) in water | -9.61           | -42.09 to 22.87 | 0.758            |
| 3% \(\text{H}_2\text{O}_2\) in saline vs 3% topical \(\text{H}_2\text{O}_2\) | 66.83           | 34.35 to 99.32  | < 0.0001*        |
| 3% \(\text{H}_2\text{O}_2\) in water vs 3% topical \(\text{H}_2\text{O}_2\) | 74.44           | 43.96 to 108.9  | < 0.0001*        |
| **Time: 20 mins**                    |                 |                 |                  |
| 3% \(\text{H}_2\text{O}_2\) in saline vs 3% \(\text{H}_2\text{O}_2\) in water | -10.39          | -42.87 to 22.1  | 0.7236           |
| 3% \(\text{H}_2\text{O}_2\) in saline vs 3% topical \(\text{H}_2\text{O}_2\) | 63.22           | 30.73 to 95.7   | < 0.0001*        |
| 3% \(\text{H}_2\text{O}_2\) in water vs 3% topical \(\text{H}_2\text{O}_2\) | 73.61           | 41.12 to 106.1  | < 0.0001*        |

*Statistically significant
CI, confidence interval

Fig. 3a
Time course of \(\text{H}_2\text{O}_2\) effect. a) Bacteria grown in tryptic soy broth (TSB) showed that a 3% topical solution is superior to the other solutions in its bactericidal effect, starting from five minutes of incubation. 3% \(\text{H}_2\text{O}_2\) prepared in saline is not significantly different from 3% \(\text{H}_2\text{O}_2\) prepared in water in any of the time-points. *\(p < 0.0001\) compared with control saline or water; †\(p < 0.0001\) compared with 3% \(\text{H}_2\text{O}_2\) prepared in saline or in water.

Fig. 3b
Bacteria grown in tryptic soy broth–defibrinated sheep blood (TSB-DSB) agar and treated with each solution of \(\text{H}_2\text{O}_2\) for five minutes was grown as lawn to further assess for growth inhibition. All treatment solutions inhibited bacterial growth except the controls with water or saline.

minutes, the 3% topical solution significantly decreases the viability of C. acnes compared with the water-only control \((p < 0.0001)\), and it was superior in its bactericidal effect compared with 3% \(\text{H}_2\text{O}_2\) prepared either in saline or water during all times analyzed \((p < 0.0001)\). Bacteria treated for five minutes with each of the tested solutions were grown as lawn to further test growth inhibition (Fig. 3b). Only negative controls showed normal growth of C. acnes as lawn. None of the treatment solutions displayed any appearance of colonies.

Bacteria stained with Live/Dead BacLight kit were viewed with a Nikon Eclipse Ti fluorescence microscope (Nikon Corp., Tokyo, Japan) to assess the staining profile and investigate the negative values of cell viability obtained for 3% topical solution. The standard curve for live/dead bacteria showed a gradual decrease in green staining (live cells), together with an increase in red staining (dead cells) (Fig. 4a). This was visible for both 3% \(\text{H}_2\text{O}_2\) in saline and in water. However, topical solutions showed a decrease of fluorescence staining for both green and red (Fig. 4b). The findings for the topical \(\text{H}_2\text{O}_2\) fluorescence staining indicate that this treatment results in not just cell death, but also complete cell destruction.
Discussion

The results of this study serve to validate the bactericidal and bacteriostatic properties of H$_2$O$_2$ against a specific bacterium, C. acnes. After treatment with H$_2$O$_2$, the colonies with C. acnes showed complete elimination of bacteria within five minutes. The oxidative reaction produced caused extensive damage and even destroyed the bacterial cells. After the treatments with H$_2$O$_2$, attempts to regrow the C. acnes produced negative results for at up to a week, a timepoint significantly longer than controls. The minimum inhibitory concentration of H$_2$O$_2$ for C. acnes is 1% solution prepared with either saline or water. More importantly, our results demonstrated that the commercially available topical solution is as effective as the 1% freshly prepared solution, indicating that it is as powerful as the lowest concentration of H$_2$O$_2$ solution. Additionally, our results found that the topical solution had a superior bactericidal effect compared with 3% H$_2$O$_2$ prepared in either saline or water, and found that the minimum time for bactericidal effect on C. acnes was five minutes.

Previous literature has shown that H$_2$O$_2$ has both bactericidal and bacteriostatic effect in vitro on other bacteria.\textsuperscript{28,30} To our knowledge, this is the first study to examine the efficacy of H$_2$O$_2$ as it pertains to C. acnes. The results of this study are powerful in that the H$_2$O$_2$ topical solution is low cost, with an average price of $1.30 (USD) for a 473.18 ml (16 ounce) bottle, and can be easily applied to the surgical field at any time during the surgical procedure. The ease of application provides a means
of delivery to the deep dermal layer where sebaceous glands reside.

This study was undertaken in order to find a more efficacious form of skin preparation prior to shoulder surgery to reduce the bacterial burden of \(C.\) \(acnes\) and decrease the risk of deep contamination and potential infection. Studies have shown that culture-positive \(C.\) \(acnes\) patients may remain positive in nearly a third following treatment with chlorhexidine, and may be even double this at the end of a surgical procedure.\(^6\) The current ‘standard of care’ skin preparations are questionably effective in eliminating \(C.\) \(acnes\) colonization, therefore alternative skin preparations must be considered in order to reduce the incidence of postoperative infection by this troublesome bacterium. Although, to date, no study has been performed that looks explicitly at the effects of \(H_2O_2\) on \(C.\) \(acnes\) colonization in vivo, there have been studies examining the cultures of shoulders after treatment with benzoyl peroxide, a similarly structured analogue. In this study, Sabetta et al\(^9\) showed that pre-treatment with benzoyl peroxide produced similarly low positive culture rates for \(C.\) \(acnes\) as a control swab, indicating the ability of the substance to penetrate the sebaceous glands and eliminate the bacterium.

A recent study by Namdari et al\(^8\) performed as a randomized controlled trial to evaluate the effectiveness of a preoperative course of oral doxycycline for seven days prior to shoulder arthroscopy to determine whether this treatment would reduce \(C.\) \(acnes\) colonization in males. The authors reported no difference in positive culture rates between the ‘no antibiotics’ and the doxycycline groups. The most alarming finding was 59.5% positive cultures in the no treatment group and 43.2% in the doxycycline group (\(p = 0.245\)). The results indicate minimal impact on \(C.\) \(acnes\) and the authors recommended against this type of prophylactic use, as there is potential risk for the emergence of resistance. These results also support the findings reported by Matsen et al\(^{15}\) and Falconer et al\(^6\) that indicate that current intravenous administered antibiotics given prior to skin incision do not eliminate \(C.\) \(acnes\). Both of these studies found a 30% culture-positive rate despite perioperative antibiotics, thus supporting the need for additional/other interventions to eradicate this bacteria successfully at the time of surgery.

While this study certainly produces meaningful information, it has somelimitations. This study is an in vitro analysis of the effects of \(H_2O_2\) on \(C.\) \(acnes\). Although the results show efficacy in the laboratory, the clinical utility has still not been investigated. In the laboratory, the \(H_2O_2\) comes into direct contact with the bacterial membranes, allowing for aggressive destruction of cells, as demonstrated by the negative curve for the topical \(H_2O_2\) solution in Figure 3a. In vitro, the bacteria tend to live in the pilosebaceous glands in the subcutaneous dermal layer of the skin, a location that is not readily accessible to most skin preparations.\(^4,13\) Another limitation is that we used only one bacterial reference strain, and while it is not common clinically to speciate bacterial strains of this organism, we do not know if clinical isolates of \(C.\) \(acnes\) would demonstrate the same susceptibility to \(H_2O_2\). The efficacy of \(H_2O_2\) is a topic for future investigation.

The use/application of \(H_2O_2\) at the time of surgery will need to be at multiple locations to be able to get in contact with the bacteria and be effective. Although this is not an issue during open surgical procedures, \(H_2O_2\) may not be as effective during arthroscopic shoulder surgery because its penetration into the deep dermal layer through topical application is unknown. While benzoyl peroxide has been shown to penetrate the pilosebaceous glands of the skin, no such study has been performed for \(H_2O_2\).\(^{14}\) It is clear that it is not possible to eradicate \(C.\) \(acnes\) completely, and the focus and goal should be to decrease the bacterial burden in order to prevent contamination of the surgical field and not allow this troublesome bacteria to establish a biofilm deep within the shoulder.

In conclusion, hydrogen peroxide is a very potent antimicrobial against the bacterium \(Cutibacterium\) \(acnes\). It is both bacteriostatic and rapidly bactericidal, even at low concentrations. The minimum bactericidal concentration of \(H_2O_2\) for \(C.\) \(acnes\) is a 1% solution prepared in either saline or water. The commercially available topical 3% solution is equally as effective as the laboratory-prepared concentration. Within five minutes of exposure to \(H_2O_2\), there is complete eradication of \(C.\) \(acnes\) in vitro. Further studies are needed to establish the depth of penetration through the epidermis to the deep dermal layers. With our current findings, the use of a topical solution of \(H_2O_2\) as part of skin preparation prior to surgery may be a helpful tool to prevent \(C.\) \(acnes\) contamination during shoulder surgery. Additionally, application to the deep dermal layer once a skin incision has been made, and a final application to the deep dermal layer prior to skin closure, may be helpful in the eradication of this troublesome bacterium. Future studies are required in order to validate these recommendations clinically.

References

1. Scholz CF, Kilian M. The natural history of cutaneous propionibacteria, and reclassification of selected species within the genus Propionibacterium to the proposed novel genera Acidipropionibacterium gen. nov., Cutibacterium gen. nov. and Pseudopropionibacterium gen. nov. \(Int J Syst Evol Microbiol\) 2016;66:4422-4432.
2. Mook WR, Klement MR, Green CL, Hazen KC, Garrigue GE. The incidence of propionibacterium acnes in open shoulder surgery: a controlled diagnostic study. \(J Bone Joint Surg [Am]\) 2015;97-A:957-963.
3. Hudek R, Sommer F, Abdel-kawi AF, et al. Propionibacterium acnes in shoulder surgery: is loss of hair protective for infection? \(J Shoulder Elbow Surg\) 2016;25:973-980.
4. Falconer TM, Baba M, Kruse LM, et al. Contamination of the surgical field with propionibacterium acnes in primary shoulder arthroplasty. \(J Bone Joint Surg [Am]\) 2016;98-A:1722-1728.
5. Dizay HH, Lau DG, Nottage WM. Benzoyl peroxide and clindamycin topical skin preparation decreases Propionibacterium acnes colonization in shoulder arthroscopy. J Shoulder Elbow Surg 2017;26:1190-1195.

6. Lee MJ, Pottinger BS, Butler-Wu S, et al. Propionibacterium persists in the skin despite standard surgical preparation. J Bone Joint Surg [Am] 2014;96-A:1447-1450.

7. Murray MR, Saltzman MD, Gryzlo SM, et al. Efficacy of preoperative home use of 2% chlorhexidine gluconate cloth before shoulder surgery. J Shoulder Elbow Surg 2011;20:926-933.

8. Namdari S, Nicholson T, Parvizi J, Ramsey M. Preparative doxycycline does not decontaminate Propionibacterium acnes from the skin of the shoulder: a randomized controlled trial. J Shoulder Elbow Surg 2017;26:1495-1499.

9. Sabetta JR, Rana VP, Vadasdi KB, et al. Efficacy of topical benzoyl peroxide on the reduction of Propionibacterium acnes during shoulder surgery. J Shoulder Elbow Surg 2015;24:995-1004.

10. Saltzman MD, Nuber GW, Gryzlo SM, et al. Antibiotic susceptibility of Propionibacterium acnes isolated from orthopaedic implant-associated infections. J Shoulder Elbow Surg 2016;25:304-310.

11. Sardana K, Gupta T, Garg VK, Ghuwanaw S. Antibiotic resistance to Propionibacterium acnes: worldwide scenario, diagnosis and management. Expert Rev Anti Infect Ther 2015;13:883-896.

12. Takoudji EM, Guillonoucou F, Kambarov S, Pecorari F, Corvec S. In vitro emergence of fluoroquinolone resistance in Cutibacterium (formerly Propionibacterium) acnes and molecular characterization of mutations in the gyrA gene. Anaerobe 2017;47:194-200.

13. Bolton JE Jr, Farzad-Bakshandeh A, Bradley S. Studies on the mechanism of action of topical benzoyl peroxide and vitamin A acid in acne vulgaris. J Cutan Pathol 1974;1:191-200.

14. Leyden JJ. Effect of topical benzoyl peroxide/clindamycin versus topical clindamycin and vehicle in the reduction of Propionibacterium acnes. Curr Med Res Opin 2002;18:475-480.

15. Leyden JJ, Del Rosso JQ, Webster GF. Clinical considerations in the treatment of acne vulgaris and other inflammatory skin disorders: focus on antibiotic resistance. Cutis 2007;79(Suppl):9-25.

16. Leyden JJ. Preoperative doxycycline does not decolonize Propionibacterium acnes from the skin of the shoulder: a randomized trial. J Shoulder Elbow Surg 2017;26:1495-1499.

17. Haider A, Shaw JC. Treatment of acne vulgaris. JAMA 2004;292:726-735.

18. Leyden JJ, Del Rosso JQ, Webster GF. Clinical considerations in the treatment of acne vulgaris and other inflammatory skin disorders: focus on antibiotic resistance. Cutis 2007;79(Suppl):9-25.

19. Craven JD, Hohmann SR, Podolsky R, et al. The penetration of benzoyl peroxide in the skin. J Bone Joint Surg [Am] 2009;91-A:1949-1953.

20. Phadnis J, Gordon D, Krishnan J, Bain GI. Efficacy of surgical preparation solutions in shoulder surgery. J Bone Joint Surg [Am] 2009;91-A:1949-1953.

21. Allborn M, Arve S, Bruggemann H, Lood R. A novel enzyme with antioxidant capacity produced by the ubiquitous skin colonizer Propionibacterium acnes. Sci Rep 2016;6:36412.

22. Leyden JJ, Del Rosso JQ, Webster GF. Clinical considerations in the treatment of acne vulgaris and other inflammatory skin disorders: focus on antibiotic resistance. Cutis 2007;79(Suppl):9-25.

23. Hyslop PA, Hinshaw DB, Sraufstatter IU, et al. Hydrogen peroxide as a potent bacteriostatic antibiotic: implications for host defense. Free Radic Biol Med 1995;19:31-37.

24. Hall GD, Pratt-Rippin K, Meisler DM, et al. Hydrogen peroxide and propylhexylostearate: implications for host defense. Free Radic Biol Med 1995;19:31-37.

25. Leyden JJ, Del Rosso JQ, Webster GF. Clinical considerations in the treatment of acne vulgaris and other inflammatory skin disorders: focus on antibiotic resistance. Cutis 2007;79(Suppl):9-25.

26. Leyden JJ, Del Rosso JQ, Webster GF. Clinical considerations in the treatment of acne vulgaris and other inflammatory skin disorders: focus on antibiotic resistance. Cutis 2007;79(Suppl):9-25.

27. Leyden JJ, Del Rosso JQ, Webster GF. Clinical considerations in the treatment of acne vulgaris and other inflammatory skin disorders: focus on antibiotic resistance. Cutis 2007;79(Suppl):9-25.

28. Leyden JJ, Del Rosso JQ, Webster GF. Clinical considerations in the treatment of acne vulgaris and other inflammatory skin disorders: focus on antibiotic resistance. Cutis 2007;79(Suppl):9-25.

29. Leyden JJ, Del Rosso JQ, Webster GF. Clinical considerations in the treatment of acne vulgaris and other inflammatory skin disorders: focus on antibiotic resistance. Cutis 2007;79(Suppl):9-25.

30. Leyden JJ, Del Rosso JQ, Webster GF. Clinical considerations in the treatment of acne vulgaris and other inflammatory skin disorders: focus on antibiotic resistance. Cutis 2007;79(Suppl):9-25.

31. Leyden JJ, Del Rosso JQ, Webster GF. Clinical considerations in the treatment of acne vulgaris and other inflammatory skin disorders: focus on antibiotic resistance. Cutis 2007;79(Suppl):9-25.

32. Leyden JJ, Del Rosso JQ, Webster GF. Clinical considerations in the treatment of acne vulgaris and other inflammatory skin disorders: focus on antibiotic resistance. Cutis 2007;79(Suppl):9-25.

33. Leyden JJ, Del Rosso JQ, Webster GF. Clinical considerations in the treatment of acne vulgaris and other inflammatory skin disorders: focus on antibiotic resistance. Cutis 2007;79(Suppl):9-25.

34. Leyden JJ, Del Rosso JQ, Webster GF. Clinical considerations in the treatment of acne vulgaris and other inflammatory skin disorders: focus on antibiotic resistance. Cutis 2007;79(Suppl):9-25.

35. Leyden JJ, Del Rosso JQ, Webster GF. Clinical considerations in the treatment of acne vulgaris and other inflammatory skin disorders: focus on antibiotic resistance. Cutis 2007;79(Suppl):9-25.