Efficacy and safety of a novel antimicrobial preoperative skin preparation

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Abstract

Objective: Alternatives to skin preparation with conventional preoperative antiseptics are required because of adverse reactions and the potential emergence of resistance. Here, we present 2 phase 2 studies of ZuraGard (ZG), a novel formulation of isopropyl alcohol and functional excipients developed for preoperative skin antisepsis.

Methods: Microbial skin flora on abdominal and inguinal sites in healthy volunteers were quantitatively assessed following application of ZG versus a negative control (ZV) and a chlorhexidine/alcohol preparation, Chloraprep (CP). In trial 1, ZG administered for both recommended and abbreviated application times was compared with CP and ZV via bacterial reductions at 10 minutes, and 6 hours, 12 hours, and 24 hours following application. In trial 2, the 10-minute postapplication responder rates (RRs) for ZG, participants with abdominal ≥2 log10 per cm², and inguinal ≥3 log10 per cm² reductions in colony-forming units (CFU) were compared to RRs of participants treated with CP.

Results: In trial 1, ZG at the recommended application time reduced mean bacterial counts by ~3.18 log10 CFU/cm² and ~2.98 log10 CFU/cm² at abdominal and inguinal sites, respectively. Qualitatively similar reductions were observed for the abbreviated ZG application time and all CP applications. Application of ZV was ineffective. In trial 2, 10-minute RRs for ZG and CP exceeded 90% at abdominal sites. At inguinal sites, RRs were 83.3% for ZG and 86.7% for CP. No skin irritation or other adverse events were observed.

Conclusions: ZG matched CP efficacy under these experimental conditions with immediate and persistent microbial reductions, including abbreviated application times. Further clinical studies of this novel preoperative antiseptic are merited.

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review boards reviewed and approved these study protocols. Healthy, adult male and female participants with skin surfaces free of tattoos, dermatoses, abrasions, cuts, lesions or other skin disorders within 15.24 cm (6 inches) of the test site were recruited at 2 US study sites. Potential participants whose bacterial counts were <1.3 × 10^3 CFU/cm^2 on the abdomen and/or 1.0 × 10^5 CFU/cm^2 on the inguinal region during pretreatment screening were excluded. Additional exclusions criteria included a history of skin allergies, skin cancer within 15.24 cm (6 inches) of the study site, or any sensitivity to natural rubber latex, adhesive skin products (eg, adhesive dressings, medical tapes), isopropyl alcohol, or any ZG excipients. Participants currently using products containing CHG were also excluded. All female participants of childbearing potential were required to have negative urine pregnancy test results prior to treatment/testing.

**Study design**

We conducted 2 phase 2 studies in which ZG was compared to CP and/or an alcohol-free ZG vehicle (ZV). Our overall aim was to assess the immediate and persistent activity of ZG against natural bacterial flora on the skin of adult human participants and to compare these outcomes with those obtained with CP as a reference and ZV as a negative control.

On the day of treatment, participants providing informed consent were assigned a unique code which specified the randomly assigned application locus of test solutions used in these studies. Participants underwent sampling to measure baseline cutaneous microbial counts at abdominal and inguinal sites prior to application of study test solutions. In each study, the primary investigator or a designated clinical team member typically applied test solutions per protocol to each test region by scrubbing over a 3.8 × 12.7-cm (1.5 × 5-inch) area at inguinal sites (rich in sebaceous glands) and over a 12.7 × 12.7-cm (5 × 5-inch) area at abdominal sites (poor in sebaceous glands). Each test site was air dried, and the posttreatment weight of the applicator was recorded. Following application of test solutions, cutaneous samples for culturing were obtained from study skin sites using the time intervals noted below.

In the first phase 2 study (trial 1), the primary outcome measure was a comparison of mean log_{10} reductions in colony-forming units per cm^2 [log_{10} CFU/cm^2)] from baseline for 2 different ZG application times (recommended and abbreviated) versus equivalent ZV and standardized CP application times. Inguinal and abdominal sites on each side of each participant’s body were randomly assigned (1) recommended ZG application times (2-minute inguinal scrub and 30-second abdominal scrub); (2) abbreviated ZG application times (1-minute inguinal scrub and 15-second abdominal scrub); (3) standardized CP application times (2-minute inguinal scrub and 30-second abdominal scrub); or (4) recommended ZV application times (2-minute inguinal scrub and 30-second abdominal scrub). Postapplication cutaneous samples were collected at 10 minutes, 6 hours, and at either 12 or 24 hours after application.

The second phase 2 study (trial 2) was designed similarly with 3 important modifications. First, a negative control was not used, and ZG and CP test solutions were compared directly against each other. Second, the abbreviated 15-second ZG application time was used only for abdominal sites; the recommended 2-minute ZG application time was used for inguinal sites. Third, the antimicrobial effectiveness of the test solutions was assessed as 10-minute responder rates (RR) where RR was the proportion of participants with a ≥2 log_{10} CFU/cm^2 reduction from baseline at the abdominal site and a ≥3 log_{10} CFU/cm^2 reduction from baseline at the inguinal site 10 minutes after test solution application. Bacterial counts at both loci were required to remain below baseline counts 6 hours later. The primary objective was to meet or exceed a 70% RR.

Immediately after the 10-minute skin sample was taken in both studies, skin testing sites on the abdomen or groin targeted for 6-hour, 12-hour, and 24-hour testing were covered with sterile, semicircular gauze taped to the skin to cover and protect the test site. All participants were instructed not to remove the dressing, to limit their physical activity, to avoid sweating, and to avoid any potential test site contamination. This procedure is in line with studies that do not house participants for the entire testing period and consistent with preoperative study designs. No washing of the product application sites and no friction with clothing occurred.

**Bacterial sampling procedures**

All microbial specimens were collected from each skin site in each study by the testing site’s clinical team using a sterile cylinder containing 3.0 mL sterile stripping and suspending fluid with product neutralizers. Once in contact with the skin, the area surrounding the cylinder was massaged to enhance collection of cutaneous flora. All samples were transferred to a sterile counting tube. A second aliquot sample was collected in the same manner immediately afterward. Both were combined and serially diluted in Butterfield’s phosphate buffer containing product neutralizers. Plated cultures were prepared from each of these dilutions on tryptic soy agar with product neutralizers (TSA+) and incubated at 30°C for ~72 hours, or until the appearance of sufficient bacterial growth. Colonies were counted on culture plates by technicians blinded to the sample origin. Cutaneous bacterial counts at baseline and at each postapplication time were recorded for each test area of skin surface in colony-forming units (CFU) per square centimeter (cm^2).

**Statistical methods**

A modified intent to treat (mITT) approach, restricted to participants with baseline cutaneous microbial counts >1.3 × 10^3 CFU/cm^2 at abdominal sites and >1.0 × 10^5 CFU/cm^2 at inguinal sites on the day of treatment was used for statistical analysis. Differences in counts between baseline and each programmed postapplication period were calculated as log_{10} CFU/cm^2 data. Descriptive statistics for bacterial count reductions were computed for each sampling site and for each postapplication assessment period using mean, median, standard deviation (SD), and minimum/maximum recovery count. In trial 2, the log recovery counts were converted to binary measures for each participant in the primary analyses to achieve a binomial distribution for RR data. The RR itself was derived from the number of successes at a given time point divided by the number of measurable values for that time point (eg, 18 successes at 24 time points = 75% RR). All results were reported as a net change and 95% confidence intervals (CI) calculated using least-square means. Minitab and the SAS version 8.2 statistical software (SAS Institute, Cary, NC) were used for all statistic calculations.

**Safety**

Safety was monitored for both studies by cutaneous reactivity at each test site. All local and systemic adverse events (AEs) observed or reported to the investigators were evaluated and followed to resolution along with intensity, duration, and causal relationship to the tested agent. Adhesive reactions as well as acute responses to the sampling techniques were likely and expected and included mild skin irritation and erythema, along with possible allergic reactions.
Results

Overall, 100 participants were enrolled and randomized. Notably, 4 participants enrolled in trial 1 who had previously met the necessary baseline cutaneous microbial count thresholds during the screening visit had microbial counts below these required thresholds on the day of treatment and were excluded from the final analyses. Consequently, 96 participants were included in the final mITT analyses.

Trial 1 participants were 47 males and 17 females aged 18–74 years. Most (ie, 52) were of European-American (white) ethnicity, 1 was African-American (black), 6 were Hispanic, 2 were Asian, and 3 self-identified as “other” ethnicities. Trial 2 participants comprised 20 males and 16 females aged 20–67 years. Furthermore, 20 participants self-identified as European-American, 2 as African-American, 2 as Hispanic, 10 as Asian, and 2 as “other.”

| Variable | Sample | N | Mean | SD | Min | Max |
|----------|--------|---|------|----|-----|-----|
| Reduction | 10 min post prep | 20 | 2.975 | 0.979 | 1.348 | 4.333 |
| 6 h post prep | 20 | 3.328 | 1.275 | 1.257 | 5.492 |
| 12 h post prep | 8 | 4.081 | 1.266 | 2.694 | 6.038 |
| 24 h post prep | 12 | 3.477 | 1.518 | 1.124 | 5.429 |

Results for ZG applied for 1 min to the inguinal site

| Variable | Sample | N | Mean | SD | Min | Max |
|----------|--------|---|------|----|-----|-----|
| Reduction | 10 min post prep | 21 | 3.061 | 1.102 | 1.239 | 5.489 |
| 6 h post prep | 21 | 3.161 | 1.116 | 1.328 | 5.334 |
| 12 h post prep | 8 | 4.326 | 1.082 | 3.012 | 5.666 |
| 24 h post prep | 13 | 3.714 | 1.682 | 1.251 | 6.280 |

Results for ZV applied for 2 min to the inguinal site

| Variable | Sample | N | Mean | SD | Min | Max |
|----------|--------|---|------|----|-----|-----|
| Reduction | 10 min post prep | 24 | 1.234 | 0.580 | 0.112 | 2.536 |
| 6 h post prep | 24 | 1.673 | 0.856 | 0.276 | 3.517 |
| 12 h post prep | 9 | 2.111 | 0.977 | 0.349 | 3.974 |
| 24 h post prep | 15 | 1.365 | 0.646 | 0.164 | 2.481 |

Results for CP applied for 2 min to the inguinal site

| Variable | Sample | N | Mean | SD | Min | Max |
|----------|--------|---|------|----|-----|-----|
| Reduction | 10 min post prep | 11 | 2.754 | 0.369 | 2.277 | 3.444 |
| 6 h post prep | 11 | 3.520 | 1.322 | 1.564 | 5.880 |
| 12 h post prep | 5 | 3.244 | 1.069 | 2.383 | 5.089 |
| 24 h post prep | 6 | 2.653 | 1.120 | 1.502 | 4.318 |

Note. SD, standard deviation; Min, minimum; Max, maximum; ZG, ZuraGard; ZV, ZuraGard vehicle; CP, Chloraprep.

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**Trial 1**

In trial 1, 60 participants were randomized and treated. We report those treated sites which passed baseline entry criteria and were included for analysis. Because the database used was acceptable sites rather than participants, there were frequently a different number of sites for each time point in each trial conducted. As shown in Table 1, 10-minute postapplication cutaneous microbial counts at inguinal sites dropped an average of 2.97 log_{10} CFU/cm^2 following ZG application for the recommended time, and an average of 3.061 log_{10} CFU/cm^2 following the abbreviated (1-minute) ZG application. Average reductions in inguinal counts remained at ≥3.0 log_{10} CFU/cm^2 at 6 hours, 12 hours, and 24 hours after ZG application. Although both applications of ZG produced greater mean reductions in microbial flora than did a 2-minute application of CP, these differences did not achieve statistical significance (Fig. 1).

As shown in Table 2, at the 10-minute postapplication period, cutaneous microbial counts at abdominal sites dropped an average of 3.181 log_{10} CFU/cm^2 following ZG application for the recommended time. A reduction of 2.707 log_{10} CFU/cm^2 followed the abbreviated (15-second) ZG application time. Average reductions in abdominal counts exceeded 2.0 log_{10} CFU/cm^2 at 6 hours, 12 hours, and 24 hours after ZG application. Application of the CP test solution resulted in a mean reduction in abdominal cutaneous microbial counts of 2.07 log_{10} CFU/cm^2 after 10 minutes. Although reductions in cutaneous microbial counts were below 2.0 log_{10} CFU/cm^2 12 hours following
application, reductions exceeding threshold were observed at 6 hours and 24 hours, respectively. Thus, although average abdominal reductions with ZG exceeded those with CP at all time points, these benchmark differences were not statistically significant (Fig. 2). Application of ZV did not achieve target reductions in cutaneous microbial counts at either the inguinal or abdominal sites.

No skin irritation or other AEs were observed before baseline, after test agent application, or before sample collection time points for all treated participants.
rapidly than do other aqueous solutions. Here, we describe the application of alcohol-containing products to pre-
pare the skin prior to any surgical procedure because they act more effectively as surgical irrigation products, and oral rinses, raise concerns about the practical significance because shorter application times have been correlated with higher levels of staff adherence to surgical site preparation protocols in at least 1 study.\(^{18}\)

In view of the potential for regrowth of skin bacteria during the surgical procedure, and given the short duration of alcohol-based antiseptic activity, guidelines recommend that agents used for surgical site preparation contain an additional component to promote prolonged residual activity, typically povidone-iodine or CHG. Although CHG may possess residual activity that is more pronounced than povidone-iodine,\(^{16,39}\) there is insufficient evidence to preferentially support one type of alcohol-containing preparation over another or to suggest that addition of another antimicrobial could contribute to initial efficacy.\(^{17,26}\) Nevertheless, alcohol and CHG combinations have become the preferred surgical site preparation in the United States.\(^{18}\) There are compelling reasons to pursue the development and evaluation of alternatives to conventional preoperative antiseptics. For example, IgE-mediated anaphylactic/anaphylactoid reactions triggered by CHG exposure,\(^{27}\) although rare, are increasingly described in association with surgical procedures.\(^{28}\) Hypersensitivity reactions mediated by other mechanisms are also well known,\(^{29}\) and given the myriad of opportunities for CHG exposure, there is a legitimate concern that the number of surgery patients with an allergy to this agent will increase.\(^{30}\)

Moreover, the burgeoning number of CHG-containing healthcare products, including medical devices, hand soaps, body washes, surgical irrigation products, and oral rinses, raise concerns about the.

### Table 3. Mean Log\(_{10}\) CFU Counts and Responder Rates at 10 Minutes\(^{a}\)

| Test Site | Test Agent | Baseline Count | 10-Min Count | 10-Min Reduction (95% CI) | 10 Min % Responder Rate (counts) |
|-----------|------------|----------------|--------------|--------------------------|----------------------------------|
| Abdominal | CP         | 3.35           | 0.48         | 2.88 (2.5017–3.1936)     | 90.00 (27/30)                    |
| Abdominal | ZG         | 3.36           | 0.28         | 3.08 (2.7036–3.3955)     | 90.00 (27/30)                    |
| Inguinal  | CP         | 5.39           | 1.28         | 4.11 (3.7464–4.4383)     | 86.67 (26/30)                    |
| Inguinal  | ZG         | 5.43           | 1.17         | 4.27 (3.9042–4.5961)     | 83.33 (25/30)                    |

Note. CFU, colony-forming units; CI, confidence interval; ZG, ZuraGard; CP, Chloraprep.

\(^{a}\)Mean Log\(_{10}\) CFU counts and responder rates at 10 min as calculated for ZG and CG in both abdominal and inguinal sites at baseline and 10 min after application. Reduction, change, and CI were calculated using least-squares means and are thus slightly different than numerical mean values.

**Discussion**

Reducing cutaneous bacterial counts at procedural sites is an effective strategy for reducing SSI risk. Existing guidelines recommend the application of alcohol-containing products to prepare the skin prior to any surgical procedure because they act more rapidly than do other aqueous solutions.\(^{16,24}\) Here, we describe the results of 2 phase 2 trials that demonstrate the effectiveness of a novel preoperative antiseptic, ZuraGard, in reducing bacterial counts on the skin of healthy volunteers. ZuraGard reduced microbial counts at both inguinal (\(\geq 3 \log_{10}\) CFU/cm\(^2\) reduction) and abdominal (\(\geq 2 \log_{10}\) CFU/cm\(^2\) reduction) test sites, caused no observable skin irritation or other AEs, and performed equivalently to CP, currently the most widely used pressurgical antiseptic skin preparation in the United States.\(^{18}\)

Perhaps most notably, ZG achieved targeted 10-minute postapplication microbial reductions regardless of whether a 1- or 2-minute application time was employed at inguinal sites or a 15- or 30-second application time was employed at abdominal sites. The reductions in cutaneous bacterial counts following application of ZG were sustained for up to 24 hours, and no skin irritation was observed. These findings may have important practical significance because shorter application times have been correlated with higher levels of staff adherence to surgical site preparation protocols in at least 1 study.\(^{18}\)

In trial 2, 36 participants were randomized and treated. At the 10-minute sampling period, abbreviated ZG abdominal application times yielded an average reduction in cutaneous microbial counts of 3.1 \(\log_{10}\) CFU/cm\(^2\) from baseline, although recommended ZG application to inguinal sites yielded an average reduction of 4.3 \(\log_{10}\) CFU/cm\(^2\) from baseline (Table 3). Standardized abdominal and inguinal CP applications yielded comparative count reductions of 2.9 and 4.1 \(\log_{10}\) CFU/cm\(^2\), respectively. Both ZG and CP applications exceeded the primary end-point objective of a 70% responder rate (see Table 3). No AEs were reported, and there was no evidence of an increase in cutaneous irritation after application of either test solution.

**Trial 2**

In trial 2, 36 participants were randomized and treated. At the 10-minute sampling period, abbreviated ZG abdominal application times yielded an average reduction in cutaneous microbial counts of 3.1 \(\log_{10}\) CFU/cm\(^2\) from baseline, although recommended ZG application to inguinal sites yielded an average reduction of 4.3 \(\log_{10}\) CFU/cm\(^2\) from baseline (Table 3). Standardized abdominal and inguinal CP applications yielded comparative count reductions of 2.9 and 4.1 \(\log_{10}\) CFU/cm\(^2\), respectively. Both ZG and CP applications exceeded the primary end-point objective of a 70% responder rate (see Table 3). No AEs were reported, and there was no evidence of an increase in cutaneous irritation after application of either test solution.

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Moreover, the burgeoning number of CHG-containing healthcare products, including medical devices, hand soaps, body washes, surgical irrigation products, and oral rinses, raise concerns about the...
potential for selection and spread of resistance to this particular biocide. Emerging CHG resistance has already been suggested in several outbreaks of healthcare-associated infections in the United States, and there are indications that high-frequency exposure to sublethal concentrations of CHG may enhance acquired resistance in organisms such as Acinetobacter spp, K. pneumoniae, and Pseudomonas spp, all of which are known for their virulence and adaptability to antibiotics. Notably, in terms of functional components used to support persistence and shelf-life, ZG contains a citric acid and sodium citrate solution as well as trace methyl- and propyl-parabens, all of which exhibit mild antimicrobial properties. Taken together, these findings strongly support the need for continued diversification of our topical antiseptic armamentarium.

In summary, ZG is a novel preoperative skin antiseptic that, in preliminary studies, reduces bacterial contamination of the skin and preforms similarly to CP. These results justify larger-scale studies to examine the effectiveness and safety of this product in randomized clinical trials and in more diverse patient populations.

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References

1. Steiner CA, Karaca Z, Moore BJ, Imshau MC, Pickens G. Surgeries in hospital-based ambulatory surgery and hospital inpatient settings, 2014: statistical brief #223. Healthcare Cost and Utilization project website. https://www.hcup-us.ahrq.gov/reports/statbriefs/sb223-Ambulatory-Inpatient-Surgeries-2014.pdf. Published 2017. Accessed July 3, 2019.

2. Anderson DJ. Surgical site infections. Infect Dis Clin North Am 2011;25:135–153.

3. Chow WB, Merkow RP, Cohen ME, Bilimoria KY, Ko CY. Association between postoperative complications and reoperation for patients undergoing geriatric surgery and the effect of reoperation on mortality. Am Surg 2012;78:1137–1142.

4. Vogel TR, Dombrovskiy VY, Lowry SF. Impact of infectious complications after elective surgery on hospital readmission and late deaths in the US Medicare population. Surg Infect 2012;13:307–311.

5. Li LT, Mills WI, White DI, et al. Causes and prevalence of unplanned readmissions after colorectal surgery: a systematic review and meta-analysis. J Am Geriatr Soc 2013;61:1175–1181.

6. Gibson A, Tevis S, Kennedy G. Readmission after delayed diagnosis of surgical site infection: a focus on prevention using the American College of Surgeons National Surgical Quality Improvement Program. Am J Surg 2014;207:832–839.

7. Merkow RP, Ju MH, Chung JW, et al. Underlying reasons associated with hospital readmission following surgery in the United States. JAMA 2015;313:483–495.

8. Kirkland KB, Briggs JP, Trivette SL, Wilkinson WE, Sexton DJ. The impact of surgical-site infections in the 1990s: attributable mortality, excess length of hospitalization, and extra costs. Infect Control Hosp Epidemiol 1999;20:725–730.

9. Kaye KS, Anderson DJ, Sloane R, et al. The effect of surgical site infection on older operative patients. J Am Geriatr Soc 2009;57:46–54.

10. Klevens RM, Edwards JR, Richards CL, et al. Estimating healthcare-associated infections and deaths in US hospitals, 2002. Public Health Rept 2007; 122:160–166.

11. Scott RD. The direct medical costs of healthcare-associated infections in US hospitals and the benefits of prevention. Centers for Disease Control and Prevention website. https://www.cdc.gov/haif/pdfs/haif/scott_costpaper.pdf. Published 2009. Accessed July 3, 2019.

12. Berrios-Torres SI, Umscheid CA, Braitler DW, et al. Centers for Disease Control and Prevention guideline for the prevention of surgical site infection, 2017. JAMA Surg 2017;152:784–791.

13. Anderson DJ, Podgorny K, Berrios-Torres SI, et al. Strategies to prevent surgical site infections in acute care hospitals: 2014 update. Infect Control Hosp Epidemiol 2014;35 suppl 2:S66–S88.

14. Allegranzi B, Bischoff P, de Jonge S, et al. New WHO recommendations on preoperative measures for surgical site infection prevention: an evidence-based global perspective. Lancet Infect Dis 2016;16:e276–e287.

15. Ban KA, Minei JP, Laronga C, et al. American College of Surgeons and Surgical Infection Society: surgical site infection guidelines, 2016 update. J Am Coll Surg 2017;224:59–74.

16. Maivald M, Chan ESY. The forgotten role of alcohol: a systematic review and meta-analysis of the clinical efficacy and perceived role of chlorhexidine in skin antisepsis. PLoS One 2012;7(9):e44277. doi: 10.1371/journal.pone.0044277.

17. Maivald M, Widmer AF. WHO recommendation for surgical skin antisepsis is premature. Lancet Infect Dis 2017;17:1023–1024.

18. Adapted from GHX Database; Global Healthcare Market Intelligence Medical/Surgical Product Schema 2017. (Independent report available from authors).

19. Madden GR, Sifri CD. Antimicrobial resistance to agents used for Staphylococcus aureus decolonization: is there a reason for concern? Curr Infect Dis Rep 2018;20:26.

20. Williamson DA, Carter GP, Howden BP. Current and emerging topical antibacterials and antiseptics: agents, action, and resistance patterns. Clin Microbiol Rev 2017;30:827–860.

21. Sharp G, Green S, Rose M. Chlorhexidine-induced anaphylaxis in surgical patients: a review of the literature. ANZ J Surg 2016;86:237–243.

22. Food and Drug Administration. Tentative final monograph for health-care antiseptic drug products. Fed Register 1994;59:58799–58800.

23. ASTM. E1173-15: standard test method for evaluation of preoperative, preanesthesia, or preinjection skin preparations. ASTM International website. https://standards.globalspec.com/std/9935734/ASTM%20E1173. Published 2015. Accessed July 3, 2019.

24. ICH harmonized tripartite guideline: guideline for good clinical practice E6 (R1) International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use website. Available from: https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/Em6/R1_Guideline.pdf. Published 1996. Accessed July 3, 2019.

25. Sidhuwa F, Itani KMF. Skin preparation before surgery: options and evidence. Surg Infect 2015;16:14–23.

26. Dunville JC, McFarlane E, Edwards P, Lipp A, Holmes A, Liu Z. Preoperative skin antiseptics for preventing surgical wound infections after clean surgery. Cochrane Database Syst Rev 2015;12(4):CD003949.

27. Garvey LH, Kroigaard M, Poulson LK, et al. IgE-mediated allergy to chlorhexidine. J Allergy Clin Immunol 2007;120:409–415.

28. Krishna MT, York M, Chin T, et al. Multi-centre retrospective analysis of anaphylaxis during general anaesthesia in the United Kingdom: aetiology and diagnostic performance of acute serum tryptase. Clin Exp Immunol 2014;178:399–404.

29. Abdallah C. Perioperative chlorhexidine allergy: Is it serious? J Anaesthesiol Clin Pharmacol 2015;31:152–154.

30. Moka E, Argyra E, Sifakas I, Vadalaouca A. Chlorhexidine: hypersensitivity and anaphylactic reactions in the perioperative setting. J Anaesthesiol Clin Pharmacol 2015;31:145–148.

31. Horner CL, Mawer D, Wilcox M, et al. Reduced susceptibility to chlorhexidine in staphylococci: is it increasing and does it matter? J Antimicrob Chemother 2012;67:2547–2559.

32. Kampf G. Increased resistance to chlorhexidine—is it time to establish an “antiseptic stewardship” initiative? J Hosp Infect 2016;94:213–227.

33. Wang JT, Sheng W-H, Wang J-L, et al. Longitudinal analysis of chlorhexidine susceptibilities of nosocomial methicillin-resistant Staphylococcus aureus isolates at a teaching hospital in Taiwan. J Antimicrob Chemother 2008;62:514–517.
34. Hardy K, Sunnucks K, Gil H, et al. Increased usage of antiseptics is associated with reduced susceptibility in clinical isolates of Staphylococcus aureus. MBio 2018;9(3):e00894–18.

35. Lee AS, Macedo-Vinas M, Francois P, et al. Impact of combined low-level mupirocin and genotypic chlorhexidine resistance on persistent methicillin-resistant Staphylococcus aureus carriage after decolonization therapy: a case-control study. Clin Infect Dis 2011;52:1422–1430.

36. Smith K, Gemmell CG, Hunter IS. The association between biocide tolerances and the presence or absence of qac genes among hospital-acquired and community-acquired MRSA isolates. J Antimicrob Chemother 2008;61:78–84.

37. Horner C, Mawer D, Wilcox M. Reduced susceptibility to chlorhexidine in staphylococci: is it increasing and does it matter? J Antimicrob Chemother 2012;67:2547–2559.

38. El-Othmani MM, Mahmoud BM, Pearson L, Xi H, Delfino K, Saleh KJ. Assessment of standardization in surgical site preparation: does a compliance-culture exist? Int Surg J 2016;3:1–10.

39. Hibbard JS, Mulberry GK, Brady AR, et al. A clinical study comparing the skin antisepsis and safety of Chloraprep, 70% isopropyl alcohol, and 2% aqueous chlorhexidine. J Infus Nurs 2002a;25:244e9.