American Journal of Infection Control 48 (2020) 522–526

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major Article

Evaluation of a benzalkonium chloride hand sanitizer in reducing transient *Staphylococcus aureus* bacterial skin contamination in health care workers

Sidney Bondurant MD [a,*], Tanya McKinney PhD [b], Liza Bondurant PhD [b], Lisa Fitzpatrick MD, MPH, FIDSA [c]

[a] *Medical Spark Biologics, University of Mississippi Medical Center, Jackson, MS*
[b] *Department of Math and Sciences, Delta State University, Cleveland, MS*
[c] *George Washington University School of Public Health, George Washington University School of Medicine, Washington, DC*

Key Words:
Hand hygiene
Staphylococcus aureus
Benzalkonium chloride
Antibacterial persistence
Alcohol sanitizer
Nosocomial infection

Background: This study was performed to evaluate the effectiveness of a new commercially available hand sanitizer using 0.12% benzalkonium chloride (BZK) as the active ingredient in reducing transient skin contamination with *Staphylococcus aureus* in health care workers (HCWs), as compared with the effectiveness of a 70% ethanol-based hand sanitizer.

Methods: Fingertip touch culture plates were obtained from 40 HCWs in which all HCWs used antimicrobial soap containing 0.6% chloroxylenol for handwashing according to the Centers for Disease Control and Prevention guidelines for the entire study, while continuing to use the 70% ethanol-based hand sanitizer according to the Centers for Disease Control and Prevention guidelines for the first week. After the first week, the test subjects used the BZK hand sanitizer in place of the ethanol sanitizer. A paired sample t test was conducted to compare the mean bacterial colonies grown from HCWs fingertips during the use of the BZK and ethanol hand sanitizer.

Results: The results showed a significant reduction in total bacterial colony counts of *S aureus* during the week of BZK use as compared with the week of 70% ethanol sanitizer use.

Conclusions: There was a significant decrease in transient *S aureus* on the fingertips of HCWs in the BZK hand sanitizer use week as compared with the 70% ethanol hand sanitizer use week.

A recent introduction to the consumer market of hand hygiene products, DAB (Three Kings Corporation, Corinth, MS), which contains 0.12% benzalkonium chloride (BZK) as its active ingredient was studied for persistence of antibacterial activity against *Staphylococcus aureus* on human skin as compared to a 63% ethanol-based hand sanitizer. That study showed significant killing of *S aureus* on the skin up to 4 hours postapplication for the BZK sanitizer, compared with essentially no persistent antibacterial activity of the ethanol sanitizer.[1]

In the March 8, 2019, *Morbidity and Mortality Weekly Report*, the Centers for Disease Control and Prevention (CDC) expressed concern about a failure of *S aureus* nosocomial infections to continue the downward rate trend that had been seen for several years. This statement was taken from that *Morbidity and Mortality Weekly Report*, "*S aureus* infections account for substantial morbidity in the United States. Despite significant reductions in health care–associated MRSA infections, progress is slowing. MSSA infections have not decreased as much in hospitals and might be increasing in the community. Adherence to CDC recommendations for preventing device- and procedure-associated infections and interrupting transmission, along with innovative interventions tailored to the needs of health care facilities (including decolonization) are needed to further prevent *S aureus* infections."[2]

Our study was designed to determine if the use of this new BZK-based hand sanitizer product was superior to, equal to, or inferior to a 70% ethanol-based hand sanitizer in the reduction of transient pathogenic staphylococci from the hands of health care workers (HCWs) in "real-world" conditions.

*Address correspondence to Sidney Bondurant, MD, Medical Spark Biologics, 4400 Old Canton Rd, Ste. 150, Jackson, MS 39211
E-mail address: sbondurant@medicalspark.com (S. Bondurant).

Conflicts of interest: The test product used in this study was furnished by Three Kings Corporation, Corinth, Mississippi. Sidney Bondurant and Lisa Fitzpatrick are paid consultants to Three Kings Corporation.

https://doi.org/10.1016/j.ajic.2019.08.030

Table 1
Descriptive statistics

Designation	Mean	N	Standard deviation	Standard error of the mean
Alcohol (total colony counts)	10.92	400	25.029	1.251
BZK (total colony counts)	6.63	400	14.931	0.747
Alcohol difference between AM and PM colony counts	0.385	200	29.9966	2.12108
BZK difference between AM and PM colony counts	0.325	200	19.05294	1.34725

BZK, benzalkonium chloride.

METHODS

Forty volunteer test subjects were recruited from HCWs at a cardiology clinic, a physical therapy clinic, a neurology/pain management clinic, a plastic surgery clinic, and a general medical clinic. Physicians, nurses, laboratory technicians, and physical therapists were all represented as test subjects and all were involved in direct patient care. There were 37 female test subjects and 3 male test subjects. All test subjects were already aware of current CDC recommendations for hand hygiene for HCWs. No attempt was made by the researchers to change the hand hygiene behavior of the test subjects during the study.

The study was designed to last 10 days (2 Monday through Friday workweeks), with all test subjects using the 70% ethanol hand sanitizer for the first week and then using the BZK product in place of the ethanol sanitizer for the second week. All test subjects continued to use 0.6% chloroxylenol antimicrobial hand soap for both weeks of the study. The BZK test product was provided by Three Kings Corporation.

The effect of each sanitizer on the staphylococcal population of test subject hands was assessed via the fingertip touch plate method. At the start of the workday, prior to use of any hand sanitizer or antimicrobial soap, microbial samples were collected by touching the fingertips of all 10 digits with gentle pressure to Mannitol Salt Agar plates (Hardy Diagnostics, Santa Maria, CA) for 5 seconds. This procedure was repeated at the end of the workday after determining that the test subject had not used hand sanitizer or antimicrobial soap for 15 minutes prior to collection of the touch plate. Plates were incubated for 48 hours at 35°C under aerobic conditions, and manual colony counts of *S aureus* colonies were conducted. The colony count for each determination was the total colony count from all 10 fingers.

The touch plate medium used was Mannitol Salt Agar. This medium was selected because it is selective and differential for the growth of staphylococci. It is selective for *S aureus* colonies because Mannitol Salt Agar plates allow growth of staphylococci while inhibiting the growth of most other bacterial species. It is differential in that *S aureus* colonies will be yellow surrounded by a yellow zone in otherwise light red colored media, whereas other staphylococci species will produce clear pink to red colonies with no color change in the media, and some micrococci that grow will produce large white to orange colonies with no color change in surrounding media.

The study protocol and informed consent document were approved by the Mississippi College institutional review board prior to the start of the study.

Data analysis

SPSS software (IBM Corporation, Armonk, NY) was used to conduct a paired sample t test. This analysis was conducted to compare the mean colony count on HCWs during the use of the BZK and ethanol hand sanitizer. The first analysis compared the difference in the number of bacterial colonies throughout the week of BZK and the week of ethanol hand sanitizer use. The second analysis compared the difference in the reduction of the number of bacterial colonies from the morning to the afternoon for the HCWs when using ethanol sanitizer versus when using BZK. Descriptive statistics for both comparisons are presented in Table 1, whereas the inferential statistics are presented in Table 2.

RESULTS

Our study showed a significant reduction in total bacterial colony counts ($t_{399} = 2.898$; $P < .01$) of *S aureus* during the week of BZK use as compared with the week of ethanol sanitizer use. Specifically, the total *S aureus* colony count for the alcohol week was 4,367 compared with a colony count of 2,653 for the BZK week. On average, BZK use among HCWs yielded 4.285 fewer bacterial colonies than ethanol sanitizer (95% confidence interval [1.378, 7.192]).

The mean colony count for the alcohol use week was 10.92. The mean colony count for the first day morning colony count of the BZK use week was 9.13. The mean colony count for the BZK use week was 6.63. The first day morning colony count for the BZK use week appears comparable to the mean number for the alcohol use week, which is what would be expected.

Figure 1 illustrates the cumulative graph of the daily colony counts for the week of BZK and alcohol use weeks. The graph demonstrates that users of alcohol had more bacteria on them than the users of BZK throughout the week. The line of best fit for the alcohol users had a rate of increase in cumulative colony counts of 11.785, whereas the BZK users rate of increase was 6.933. This indicates that the colony counts increased at a consistently higher rate on alcohol users than on the BZK users.

On average, HCWs who used ethanol sanitizer had 0.06 fewer bacterial colonies in the afternoon than they did in the morning

Table 2
Inferential statistics

Designation	Mean	Standard deviation	Standard error of the mean	95% confidence interval of the difference		t	*df*	Significance (2-tailed) *P* value
				Lower	Upper			
Alcohol vs BZK total colony counts	4.285	29.576	1.479	1.378	7.192	2.898	399	<.01
Alcohol vs BZK difference between AM and PM colony counts	0.06	32.98814	2.33261	−4.53981	4.65981	0.026	199	.98

BZK, benzalkonium chloride.

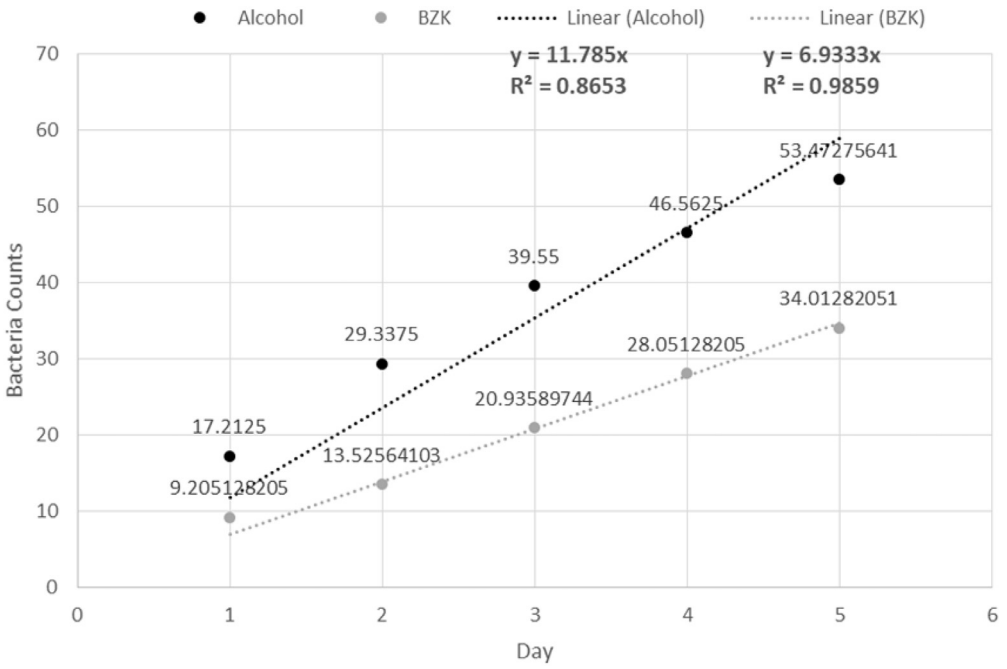

Fig 1. Cumulative bacteria counts. *BZK*, benzalkonium chloride.

compared with HCWs who used BZK (95% confidence interval [−4.53981, 4.65981]). The results did not show a significant difference between the morning and afternoon bacterial colony counts of *S aureus* of HCWs who used BZK compared with HCWs who used ethanol sanitizer ($t_{199} = 0.026$; $P > .05$).

DISCUSSION

Hand hygiene compliance is widely recognized as playing a major role in the prevention of hospital-acquired infections (HAI), and is incorporated in the CDC recommendations for preventing HAI. Despite this, compliance with the CDC guidelines is quite variable and, in some cases, very low. One recent review article of studies of hand hygiene compliance in hospital emergency departments showed that only 33% of the studies showed compliance of >50%.[3] Hand hygiene products that increase compliance should result in lower bacterial loads on the hands of HCWs.

Alcohol-based hand sanitizers (ABHS) and antiseptic hand soap for handwashing are 2 components of the current guidelines for hand hygiene for HCWs recommended by CDC.[4] The monograph used as the basis for these guidelines was published in 2002 and was an extensive review of the data available up to that time. In the section discussing BZK and other quaternary ammonium compounds the authors stated, "Further studies of such products are needed to determine if newer formulations are effective in health care settings."[5] Two areas of interest that would promote further reductions in the bacterial load on the hands would be the effect of persistent antibacterial activity of a hand sanitizer on the skin, and measures that would increase the likelihood of HCWs using the sanitizer as recommended by CDC.

BZK has been used as a hand hygiene antimicrobial for almost 90 years. It has a long history of use in both surface disinfectants used in the food industry and as a skin sanitizer. The mechanism of action for BZK is related to its ability to become adsorbed to and then penetrate the bacterial cell wall that leads to damage and loss of cell membrane structural integrity. This causes leakage of low molecular weight components of the cell and eventually cell wall lysis.[6] Alcohol is effective at killing bacteria by its ability to denature proteins. Concentrations between 60% and 95% are most effective, but higher concentrations actually lose potency because of the necessity to have water with the alcohol to be effective.[5]

Recent reports of increased tolerance to alcohol by certain pathogens have caused concern about the possibility of decreasing effectiveness of hand sanitizers.[7] Quaternary ammonium compounds such as BZK are widely used in the food industry as disinfectants, and have been studied in that context for findings of resistance to those compounds. Holah et al[8] compared *Listeria monocytogenes* and *Escherichia coli* populations taken from fish cannery lines in which 1 area was routinely disinfected with quaternary ammonium compounds and another area that had no exposure to those disinfectants. Their conclusion was that the persistent colonies found in disinfectant exposed areas were there because of factors other than tolerance to the disinfectant, primarily physical factors such as biofilm formation and surface attachment.[8] Another study found increased tolerance to BZK from some species (*Pseudomonas aeruginosa*) recovered from river sludge, whereas other species (*Klebsiella michiganensis*) from the same sample showed no increased tolerance. The basis for the difference was found to be a small change in the antibiotic efflux gene sequence.[9] Moreover, an additional study in the food industry of *L monocytogenes* found that at very low concentrations BZK did promote tolerance but at concentrations normally used the disinfectant was still very efficient at controlling this organism.[10] He et al[11] cultured inanimate objects from fitness centers and school dormitories for staphylococci. In areas where BZK antiseptics using different products with BZK concentrations ranging from 0.02%-0.12% were used, they found that 23.51% of the isolates were resistant to BZK.[11] These are not surprising findings. Sublethal concentrations of the disinfectant would allow the already tolerant subpopulation to thrive and then predominate. Lethal concentrations would kill effectively and leave the surviving fraction of the population only in low numbers.

The frequent use of ABHS can result in skin dryness and irritation, an irritant contact dermatitis. The addition of humectants and emollients to the ABHS products can help protect against this but even with these protections the use of ABHS can cause skin burning if

there is skin cracking or irritation already present on the user's hands. Both ABHS use and frequent handwashing with detergent/soap and water can cause skin cracking and irritation because of those agents' ability to denature skin proteins and to remove natural lipids on the skin that normally act to protect the skin. The effectiveness of the lipid dissolving property of alcohols is directly related to the alcohol concentration of the ABHS product.[12]

The BZK product used in this study is a new consumer product using a patent-pending formulation of BZK and inactive ingredients. The product is nonirritating, nonflammable, nonsticky, odorless, and is dispensed as a dose of 0.75 mL liquid that is converted to foam as it is dispensed. The manufacturing of BZK has changed over the years with improvements in ingredient purity. The sanitizer used in this study uses that improved purity ingredient. Previous studies have shown that HCWs using hand sanitizers prefer "fast absorption, soft/moisturized hand feel, not sticky, clean feel, and low smell" and that foam products are the preferred vehicle for delivery of the antimicrobial agent.[13]

The concentration of BZK found in the test product (0.12 %) makes it relatively nontoxic. The test product is also nondamaging to surfaces. According to the National Institute for Occupational Safety and Health, the lowest published oral toxic dose of BZK for a human is 266 mg/Kg.[14] For a 10 Kg child to ingest this amount would require drinking about 2.25 L of the test product. In contrast, alcohol hand sanitizers may be quite toxic to children in very small amounts. For the first 4 months of 2019 there were 5,829 exposure cases regarding hand sanitizers in children 12 years and younger managed by American poison control centers. Tiny amounts of alcohol hand sanitizer, such as licking a hand immediately after application of the sanitizer, would be unlikely to cause any illness but a child ingesting any amount more than just a taste would be at risk for alcohol poisoning. Alcohol poisoning may cause confusion, vomiting, drowsiness, respiratory depression, and in severe cases death. As little as 30 mL may be fatal in a small child.[15,16]

With the awareness of CDC concern about *S aureus* nosocomial infection rates at a plateau, and the problem of low compliance with hand hygiene protocols, we wanted to evaluate if replacing an ABHS with the test product would affect transient hand contamination with *S aureus*. Our results showed a mean colony count for *S aureus* of 10.92 during the medicated soap/alcohol use week. This count is consistent with the number of *S aureus* colony forming units found on the hands of HCWs in a previous study by Pittet et al,[17] therefore, we believed that our test subject population was representative. Because the BZK test product has a known persistence on human skin for up to 4 hours,[1] we theorized that there would be a decrease in the colony count on the afternoon plate from the morning plate as HCWs used BZK throughout the day. We also theorized that there would be a smaller decrease in the colony count in the ethanol week afternoon plate because of the known lack of persistent antimicrobial activity of ethanol. Neither theorized outcome was shown by the data in this study.

During the ethanol use week the morning colony count and the afternoon colony count showed no significant difference, and the BZK week showed the same result. We found the total colony count in the BZK week was significantly lower than the total colony count in the ethanol week. This may reflect the persistence of BZK on the skin for a longer time than has been previously documented. Ethanol sanitizer has an immediate kill effect on bacteria but then has no persistence. Repeated use of the ethanol sanitizer would kill bacteria present on the skin but would not prevent new bacteria from lodging on the skin surface when the test subject touched a contaminated object or person. The BZK test product is known to be effective at killing *S aureus* and maintains this killing effect for at least 4 hours, but the time the killing effect begins to wane is unknown. Another possibility could be that the "user friendly" BZK test product could have encouraged better hand hygiene compliance, and thus more killing of transient bacteria. Having fewer pathogenic transient bacteria on the hands of HCWs would provide less opportunity for the development of HAI.

Because of the many positive attributes of ABHS it is not expected that ABHS will be replaced anytime in the foreseeable future in the hand hygiene protocol recommended by the CDC. However, the negative dermatological and esthetic attributes of ABHS may be a significant factor contributing to low compliance with the CDC recommended hand hygiene protocol. Another study is planned in which the test product will be added as a "supplement" to the CDC recommended alcohol hand sanitizer plus medicated soap protocol. This planned study will add the BZK test product to the facility area where hand soap dispensers are located with recommendations for the user to apply the test product after drying the hands. This will be done in an inpatient facility to see if such use of the BZK test product can result in persistent decreased *S aureus* population on the hands of HCWs in a 2-week study, and in decreased nosocomial infection rates in a longer term study.

Limitations

The limitations to our study were that the study population was small at 40 test subjects, there was no attempt to observe or document compliance with hand hygiene protocols, there was a predominance of female test subjects, the majority of test subjects were working in outpatient facilities only, and the study was limited to evaluation of only 1 pathogenic bacteria species.

CONCLUSIONS

Use of a new "user friendly" formulation of BZK hand sanitizer that also demonstrated persistence of the BZK on the skin reduced fingertip contamination by *S aureus* in HCWs significantly as measured by colony counts. Despite the limitations of the study, the results are promising and demonstrate significant reductions in *S aureus* hand contamination can be achieved relative to alcohol. Our study findings warrant consideration in modifying hand hygiene protocols to address the problem of nosocomial infections from *S aureus*.

Acknowledgements

The authors would like to thank Diane Tryner, RN, for her technical assistance and John W. Harbell, PhD, for his most helpful comments.

References

1. Bondurant SW, Duly C, Harbell JW. Demonstrating the persistent antibacterial efficacy of a hand sanitizer containing benzalkonium chloride on human skin at 1, 2, and 4 hours after application. Am J Infect Control 2019;47:928-32.
2. Kourtis AP, Hatfield K, Baggs J, Mu Y, See I, Epson E, et al. Vital signs: epidemiology and recent trends in methicillin-resistant and in methicillin-susceptible *Staphylococcus aureus* bloodstream infections—United States. MMWR Morb Mortal Wkly Rep 2019;68:214-9.
3. Seo H-J, Sohng K-Y, Chang SO, Chaung SK, Won JS, Choi M-J. Interventions to improve hand hygiene compliance in emergency departments: a systematic review. J Hosp Infect 2019;102:394-406.
4. Centers for Disease Control and Prevention. Healthcare providers. Clean hands count for healthcare providers. Available from: https://www.cdc.gov/handhygiene/providers/index.html. Accessed May 27, 2019.
5. Boyce JM, Pittet D. Guideline for hand hygiene in health-care settings. Recommendations of the healthcare infection control practices advisory committee and the HICPAC/SHEA/APIC/IDSA hand hygiene task force. Available from: https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5116a1.htm. Accessed May 27, 2019.
6. McDonnel G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance [published correction appears in Clin Microbiol Rev 2001 Jan; 14(1):227]. Clin Microbiol Rev 1999;12:147-79.

7. Pidot SJ, Gao W, Buultjens AH, Monk IR, Guerillot R, Cater GP, et al. Increasing tolerance of hospital *Enterococcus faecium* to hand wash alcohols. Sci Transl Med 2018;10:eaar6115.
8. Holah JT, Taylor JH, Dawson DJ, Hall KE. Biocide use in the food industry and the disinfectant resistance of persistent strains of *Listeria monocytogenes* and *Escherichia coli*. J Appl Microbiol 2002;92(Suppl):111-36.
9. Kim M, Weigand MR, Og S, Hiatt JK, Krishnan R, Tezel U, et al. Widely used benzalkonium chloride disinfectants can promote antibiotic resistance. Appl Environ Microbiol 2018;84:e01202-18.
10. Kastbjerg VG, Gram L. Industrial disinfectants do not select for resistance in *Listeria monocytogenes* following long term exposure. Int J Food Microbiol 2012;160:11-25.
11. He G-X, Landry M, Chen H, Thorpe C, Walsh D, Varela M, et al. Detection of benzalkonium chloride resistance in community environmental isolates of staphylococci. J Med Microbiol 2014;63:735-41.
12. WHO guidelines on hand hygiene in health care: first global patient safety challenge clean care is safer care. Geneva: World Health Organization; 2009, p. 14. Skin reactions related to hand hygiene. Available from: https://www.ncbi.nlm.nih.gov/books/NBK144008/. Accessed May 27, 2019.
13. Greenway R, Ormandy K, Fellows C, Hollowood T. Impact of hand sanitizer format (gel/foam/liquid) and dose amount on its sensory properties and acceptability for improving hand hygiene compliance. J Hosp Infect 2018;100:195-201.
14. Centers for Disease Control and Prevention. Ammonium, alkyldimethylbenzyl-, chloride. National Institute for Occupational Safety and Health. Registry of toxic effects of chemical substances. Available from: https://www.cdc.gov/niosh-rtecs/bo3010b0.html. Accessed May 27, 2019.
15. American Association of Poison Control Centers. "Hand Sanitizer."Available from: https://aapcc.org/track/hand-sanitizer. Accessed May 27, 2019.
16. Upstate New York Poison Center. Hand sanitizing gels: a poisoning risk for children. Available from: http://www.upstate.edu/poison/pdf/community/pp_info_sheets/websht_hand-sanitizers.pdf. Accessed May 27, 2019.
17. Pittet D, Dharan S, Toureneau S, Sauvan V, Perneger T. Bacterial contamination of the hands of hospital staff during routine patient care. Arch Internal Med 1999;159:821-6.