

Evaluation of Persistent Antimicrobial Effects of an Antimicrobial Formulation

Michael S. Ferrara, PhD, ATC, FNATA*; Ron Courson, ATC, PT†; Daryl S. Paulson, PhD‡

*Department of Kinesiology, †Athletic Association, University of Georgia, Athens; ‡BioScience Laboratories, Inc, Bozeman, MT

Context: Community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) is becoming more prevalent in healthy athletic populations. Various preventive measures have been proposed, but few researchers have evaluated the protective effects of a prophylactic application of a commercially available product.

Objective: To compare the persistent antimicrobial properties of a commercially available antimicrobial product containing 4% chlorhexidine gluconate (Hibiclens) with those of a mild, nonmedicated soap (Dr. Bronner's Magic Soap).

Design: Cross-sectional study.

Setting: Microbiology laboratory, contract research organization.

Patients or Other Participants: Twenty healthy human volunteers.

Intervention(s): The test and control products were randomly assigned and applied to both forearms of each participant.

Each forearm was washed for 2 minutes with the test or control product, rinsed, and dried. At 1, 2, and 4 hours after application, each forearm was exposed to MRSA for approximately 30 minutes.

Main Outcome Measure(s): Differences in numbers of MRSA recovered from each forearm, test and control, at each postapplication time point were compared.

Results: Fewer MRSA ($P < .0001$) were recovered from the forearms treated with the test product (4% chlorhexidine gluconate) than from the forearms treated with the control product (nonmedicated soap).

Conclusions: The 4% chlorhexidine gluconate product demonstrated persistent bactericidal activity versus MRSA for up to 4 hours after application.

Key Words: methicillin-resistant *Staphylococcus aureus*, chlorhexidine gluconate, skin infections

Key Points

- Compared with a nonmedicated soap, the product containing 4% chlorhexidine gluconate was more effective in reducing the number of methicillin-resistant *Staphylococcus aureus* colonies on the forearm.
- This bactericidal action lasted at least 4 hours.

Staphylococcus aureus, often simply called “staph,” is a potentially pathogenic species of bacteria commonly carried on the skin and in the noses of healthy people¹ and is one of the most common causes of skin infections in the United States. Most are minor skin and soft tissue infections in the form of pimples, pustules, or boils. These skin lesions may be red, swollen, and painful, often with pus. Most can be treated effectively without antibiotics, but some may progress to quite serious infections if not properly evaluated and treated. In the past, such infections could be treated effectively with penicillin and related antibiotics. In recent years, however, infections due to *Staphylococcus aureus* are often resistant to penicillins and related antibiotics. Such strains, commonly called methicillin-resistant *Staphylococcus aureus* (MRSA), can be found on the skin and in the noses of some asymptomatic people. Such people are referred to as being colonized with MRSA. The noses of approximately 32% of the population are colonized with staph bacteria, of which about 0.8% at any given time are strains of MRSA.² Infection can occur when the MRSA is introduced into the body via the skin or systemically from colonized sites or via external contamination. If the source of infection cannot be

associated with a health care facility, the infection is considered community-associated MRSA (CA-MRSA). Currently, CA-MRSA is among the most common cause of skin and soft tissue infections in adults presenting at hospital emergency departments.³

Skin and soft tissue infections from CA-MRSA are emerging as a serious concern in athletic training rooms and medical clinics. Those infected are generally healthy and do not possess the risk factors typically associated with MRSA infections.⁴ Numerous case studies^{5–9} document outbreaks of CA-MRSA infections among athletes in a variety of sports at the professional, intercollegiate, and interscholastic levels of competition. Romano et al¹⁰ stated that the incidence of CA-MRSA among the players on one intercollegiate football team over 3 years ranged from 0.1% to 10.3%. Others¹¹ have reported higher and lower case rates in a variety of other sports.

Preventive measures have been identified to reduce the rate of CA-MRSA, including recommendations from the Centers for Disease Control and Prevention,¹² such as appropriate hand hygiene, gloving, use of personal protective devices, gowning, and appropriate handling of patient care equipment, instrument,

devices, and laundry. Many athletic trainers, physicians, and coaches have educated themselves and instituted appropriate preventive measures to reduce the occurrence of CA-MRSA and protect contact-sport athletes.

A number of products that aid in the prevention of CA-MRSA transmission among people and facilities are now commercially available. Because CA-MRSA is known to be readily transmitted from person to person, we sought to investigate the potential preventive effects of washing with a 4% chlorhexidine gluconate (CHG) product. Our specific objective was to determine the persistent antimicrobial properties of the CHG (test) product used as a wash when compared with a nonmedicated (control) soap at 3 time points after product application.

METHODS

Participants

Thirty-two healthy volunteers were recruited for the study. All read and signed the informed consent approved by the institutional review board, which also approved the study. Twelve recruits did not take part because they had a scheduling conflict, did not meet the inclusion criteria or met the exclusion criteria, failed to appear at the laboratory, or were not needed because the required number of participants was already enrolled. All participants possessed both hands and were free of dermatoses, cuts, lesions, hangnails, or other skin disorders on the hands and forearms. In addition, they had not used topical or systemic antimicrobials, antibiotics, or steroids for the 7-day pretest conditioning period and abstained from use of these materials until the study ended. Participants' hands and forearms were not exposed to strong detergents, solvents, other irritants, antimicrobial agents, medicated soaps, medicated shampoos, hair mousses, medicated lotions, biocide-treated pools or hot tubs, or use of tanning beds during the 7-day pretest conditioning period or on the test day. They had no known allergies to latex (rubber), alcohols, common antibacterial agents found in soaps or lotions (eg, CHG), or topical antibiotic ointments (eg, Neosporin [neomycin, bacitracin, and polymyxin B]; Johnson & Johnson Consumer Products Company, New Brunswick, NJ). No participants had a medical diagnosis of a physical condition, such as a current or recent severe illness, asthma, diabetes, hepatitis, an organ transplant, acquired immune deficiency syndrome (or human immunodeficiency virus), any immunocompromising disease, or mitral valve prolapse or needed antibiotics before dental procedures. Female participants were not pregnant or nursing a child during the pretest or test periods of the study. Participants were not screened before testing for skin colonization of the forearm by *Staphylococcus aureus*.

Twenty people completed the testing; demographic data are presented in Table 1. No adverse events, such as a rash or an infection, were reported during or after the study.

Procedures

Before testing, we performed a neutralization assay to confirm that the neutralizing solution to be used in testing would effectively halt the antimicrobial activity of the test product and the control product (if any) and that it would not be toxic to MRSA. The assay was performed as prescribed by the guidelines provided in ASTM E 1054-02, "Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents" ([http://](http://www.astm.org/DATABASE.CART/HISTORICAL/E1054-02.htm)

Table 1. Participant Characteristics (N = 20)

Demographic Variable	Value or No.
Age, y	
Minimum	19
Median	52
Maximum	74
Sex	
Male	3
Female	17
Race	
White	20
Other	0

www.astm.org/DATABASE.CART/HISTORICAL/E1054-02.htm). The neutralizing solution effectively neutralized the activity of the test and control products and was nontoxic to MRSA.

The anterior surface skin of each participant's forearms between the wrist and the antecubital fossa was used in testing. The test product (Hibiclens, lot 700020; Molnlycke Health Care, Norcross, GA) was randomly assigned to one forearm and the control product (Dr. Bronner's Magic Soap, lot 6209362; Escondido, CA) was assigned to the other. Additionally, test sites on the forearms were randomly assigned for microbial challenge 1 hour \pm 15 minutes, 2 hours \pm 15 minutes, and 4 hours \pm 15 minutes after the product was applied. The same randomized assignment applied to the left and right arms of each participant. The temperature of the water used in all procedures was controlled at $40 \pm 2^\circ\text{C}$. A technician wearing examination gloves washed the forearm skin with 5 mL of a product for 2 minutes, using up-and-down strokes with moderate pressure and speed. After the 2-minute wash, the participant rinsed the forearm until the product lather was removed and then lightly patted the skin dry with a clean, nonsterile paper towel.

The challenge suspension of *Staphylococcus aureus* (MRSA, ATCC #33593) was prepared by transferring bacterial colonies from solid media into test tubes containing a phosphate buffer solution. The resulting suspension concentration of approximately 1.0×10^9 bacteria/mL was adjusted by serial dilution to a final challenge inoculum of approximately 1.0×10^6 bacteria/mL.

Three test sites (upper, middle, and lower sites) were demarcated on the anterior surface skin of each forearm. At each postapplication time, 10 μL (0.01 mL) of challenge suspension, 1.0×10^4 bacteria, was transferred to a site, and a sterile glass rod was used to distribute the inoculum over an area approximately 1.1 cm in diameter. The site was exposed to the MRSA challenge suspension for 30 minutes (\pm 1 minute), after which the site was sampled to determine the number of MRSA that remained. This procedure was performed at 1 hour, 2 hours, and 4 hours after application of the test or control product, as follows.

A sterile cylinder with an inner cross-sectional area of 3.46 cm^2 was held firmly on the test site to be sampled. Then 2.5 mL of sterile stripping suspension fluid with product neutralizers was instilled into the cylinder, and the skin area was massaged in a circumferential manner for 1 minute. The 2.5 mL of sterile stripping suspension fluid was removed with a pipette and transferred to a sterile test tube. The process was repeated again for a second sample, which was pooled with the first for analysis. The sampled site was then decontaminated using 70% alcohol; the other sites were not exposed to alcohol.

during decontamination. The contamination, sampling, and decontamination procedure was performed on both treated forearms at 1 hour, 2 hours, and 4 hours after product application. On completion of all sampling, the forearms were thoroughly decontaminated using 70% alcohol and a scrub with a surgical handwash product.

Data Analysis

The samples were spread or spiral plated (or both) on a selective agar medium (mannitol salt agar) for incubation at $30 \pm 2^\circ\text{C}$ for approximately 48 hours. Colonies of MRSA were counted and data recorded using the QCount system (Advanced Instruments, Inc., Norwood, MA). Descriptive statistics were used to process and express the colony-forming unit (CFU) counts. These data were converted to numbers of bacteria per square centimeter and linearized by conversion to \log_{10} scale, as follows:

$$R = \log_{10} \left[\frac{F \left(\frac{\sum c_i}{n} \right) 10^{-D}}{A} \right]$$

where

R = the average number of bacteria in \log_{10} scale per square centimeter of sampling surface

F = milliliters of sterile stripping suspension fluid used for the sampling; F = 5 mL

$\frac{\sum c_i}{n}$ = average of the duplicate colony counts used for each sample collected

D = dilution factor of the plate counts

A = inner cross-sectional area of the cylinder in square centimeters; A = 3.46 cm^2

The differences between the mean populations of MRSA recovered from the test and control sites on the forearms at each postapplication time point were then calculated. To assess the statistical significance of the differences, a 2-factor analysis of variance was applied to the data with statistical significance set at $\alpha < .05$.

RESULTS

Fewer MRSA were recovered from forearms treated with CHG than with the control product (Table 2). In fact, surviving bacteria were more than 95% fewer at all 3 posttreatment time points. The percentage difference was calculated using the formula $1 - ([\text{antilog of mean } \log_{10} \text{ CFU/cm}^2 \text{ recovered for Hibiclens}] / [\text{antilog of mean } \log_{10} \text{ CFU/cm}^2 \text{ recovered for control product}]) \times 100\%$. Statistical analysis demonstrated that the numbers of bacteria recovered from forearms treated with the test product were not different ($F_{2,114} = 1.46$, $P = .236$) at each of the posttreatment samplings. Thus, the bactericidal persistence of the CHG did not decline, at least for 4 hours. In comparing the performance of the 2 products, we found that the numbers of bacteria surviving after exposure to forearms treated with CHG (mean = $1.67 \log_{10}$) were fewer ($F_{1,114} = 192.24$, $P < 0.0001$) than on forearms treated with the control product (mean = $3.23 \log_{10}$).

Table 2. Mean \log_{10} Recovery of Methicillin-Resistant *Staphylococcus aureus*

Postapplication Time, h	Condition	Mean \log_{10} Recovery	Mean Difference
1	Control	3.27	1.37
	Test	1.90	
2	Control	3.21	1.67
	Test	1.55	
4	Control	3.21	1.65
	Test	1.56	

DISCUSSION

In summary, fewer MRSA were recovered from the forearms treated with the 4% CHG solution than from the forearms treated with the control product. In fact, fewer than 5% of bacteria survived at all 3 posttreatment time points. Therefore, compared with that of a commercially available, nonantimicrobial soap, the antimicrobial activity of CHG was highly effective and persisted for up to 4 hours after application.

Very few authors have evaluated the persistent prophylactic effectiveness of an antimicrobial wash product against a potential pathogen such as MRSA. We found that a single 2-minute application of a 4% CHG topical antiseptic product protected against MRSA for up to 4 hours after exposure. A 2-minute application is thought to best represent the time it would take to wash body sites (ie, soaping to rinsing) and is considered the industry standard.¹³ The 5-mL amount of Hibiclens was based on the label instructions for a handwash. Products that contain CHG at concentrations of 2% to 4% produce rapid, immediate killing of bacteria, but as this study has shown, they also provide significant persistent bactericidal activity. Furthermore, CHG has residual (cumulative) properties. That is, CHG is retained on the epidermis, and when it is used repeatedly over time, its bactericidal activity increases.¹⁴

Recently, the National Athletic Trainers' Association (NATA)¹⁵ published a position statement on skin diseases, which stated that the evaluation for possible CA-MRSA should include a thorough history, noting the appearance of the lesion, and a culture of the lesion. Antibiotic treatment should be started immediately and the patient monitored for treatment effectiveness.

Community-associated MRSA is well known to cause skin infections that can be rapidly invasive and difficult to treat, sometimes progressing to severe systemic illness, such as osteomyelitis, pneumonia, and even death. Accordingly, athletic trainers must monitor their patients with infected skin lesions carefully for changes that may indicate progression, such as elevated temperature, pulse, or blood pressure; spreading induration and erythema; or painful, swollen regional lymph nodes. Indurated skin that is red, inflamed, thickened, tender, and shiny in appearance should raise immediate concern. To monitor the progression of induration, the athletic trainer can outline the area with a marking pen and observe it for change several times daily. The athlete also can be advised to monitor the lesion.

The findings from this study suggest numerous practical applications for the athletic trainer. Handwashing is often stated to be the most effective infection control practice that health care workers can perform.^{12,15} Therefore, athletic trainers and all health care providers must wash their hands frequently, particularly between treatments of individual athletes. Not only is the frequency of handwashing important, but the method of

washing is critical also: rubbing circles around the wrist, moving to the palms and back of hands, and then with the fingers interlaced, along the thumb, thumb webbing, and first phalanx, ending with the fingernails and cuticles. Consistent use of a product containing 4% CHG may provide longer-lasting protection from infectious bacteria because of persistent antimicrobial activity. The athletic trainer may consider using 4% CHG at periodic intervals throughout the workday (eg, start of work, midday, end of work). Because the hands are a primary site for transferring CA-MRSA, regular use of an antimicrobial soap could protect both the clinician and patients from potential infections.

Few adverse effects are associated with the use of 4% CHG in the treatment of CA-MRSA, but if contact dermatitis appears, the product should be discontinued. The product should not be used in wounds that involve more than the superficial layer of skin (eg, puncture wounds or deep lacerations). Furthermore, a 4% CHG solution should not be used on the genitals or in the eyes, ears, or mouth.

Additional Clinical Applications

Athletic trainers care for patients with a wide variety of skin lesions, including open blisters, lacerations, avulsions, surgical incisions, abrasions, and soft tissue infections. Therefore, cleansing wounds and infections is a common task. Typically, water, sterile saline, or an antimicrobial skin cleanser (or a combination of these) is used. A product containing 4% CHG may be effective for treating wounds and skin lesions infected with MRSA.

Education is a primary role of the athletic training staff, and regular in-service sessions on infectious diseases can help reduce the rate of infection in the athletic health care facility. An infection control plan should be developed and include education of all athletic and administrative personnel and custodial staff. Athletes should be educated about CA-MRSA and receive information on the appearance, associated symptoms, reporting procedures, and risk factors of staph infection. Athletes should also be taught the appropriate use of medications, including the need to follow dosage instructions carefully; to complete the entire course of treatment, even if the infection is getting better (unless instructed otherwise by a physician); never to share their medication with other people; and never to save unfinished medication for later use. An athlete education sheet is an excellent method for disseminating this information and for emphasizing appropriate preventive measures, such as practicing good general hygiene.

Thorough education of equipment managers and custodial staff members is also very important to an infection control program. Clothing must be washed with effective sanitizing detergents at the warmest water temperature possible, as recommended by the Centers for Disease Control and Prevention.¹⁶ Hard surface equipment, such as shoulder pads and helmets, and lockers, toilets, and the floors and walls of the locker room and showers areas should be cleaned routinely with an effective disinfectant.¹⁶ Because fibers tend to attract and retain viable bacteria, carpeting is a potential bacterial reservoir. Water-extraction carpet cleaning with liquid antimicrobial cleaning solution is a good option for deep cleaning, as is steam cleaning. Between deep cleanings, the custodial staff should use vacuums that incorporate a high-efficiency particulate air filter to remove 99.97% of all particles 0.3 microns and larger.¹⁷

Limitations

We did not test any other antibacterial or antimicrobial products, so our results are limited to the 2 products assessed. In addition, the evaluation period lasted only 4 hours. Whether antimicrobial activity persists beyond 4 hours is unknown.

CONCLUSIONS

Everyone has bacteria on their skin. We showed that a 4% CHG product can provide persistent protection from infectious bacteria for up to 4 hours after use. Breaks in the skin provide potential entry for opportunistic bacteria such as *Staphylococcus*. The recently published NATA position statement on skin disease¹⁵ lists detailed information on the background of MRSA, clinical features, diagnosis, treatment, prevention, and return-to-play guidelines. A comprehensive hygiene plan, use of a 4% CHG product, and proper recognition, diagnosis, and treatment of CA-MRSA will help to minimize the adverse effects of this condition.

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Address correspondence to Michael S. Ferrara, PhD, ATC, FNATA, Department of Kinesiology, University of Georgia, 300 River Road, Athens, GA 30602. Address e-mail to mferrara@uga.edu