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Major Article

Reduction of bacterial burden by copper alloys on high-touch athletic center surfaces

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Background: Athletic centers have been locations for the transmission of community-acquired infections. This study assessed the capacity of copper alloys to reduce the bacterial burden associated with high-touch athletic center equipment. Copper alloy weights and grips were rotated with rubber coated and stainless steel controls in an undergraduate college athletic center over a 16-month period. The athletic center is used by college athletic teams, student body, and local community.

Methods: The primary outcome was to compare bacterial burdens on copper and control grips by swabbing surfaces. Significance was determined using the nonparametric Mann-Whitney *U* test with significance assessed at $P < .05$. Secondary outcomes included characterizing bacterial communities on surfaces and conducting antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method.

Results: Control athletic center components carried bacterial loads 94% larger than those found on copper alloy components. Bacterial community characterization revealed *Staphylococcus* to be the most common bacterial genus found on grip surfaces. Antibiotic resistance testing of the *Staphylococcus* isolates revealed that all isolates were susceptible to vancomycin and linezolid, whereas 35% of copper alloy isolates and 44% of control isolates were resistant to erythromycin.

Conclusions: Copper alloys can mitigate the bacterial burden on high-touch surfaces. Strategically placing copper alloys in areas of high human contact can augment infection control efforts and potentially decrease community-acquired infections in athletic centers.

According to the World Health Organization, the rise of antibiotic-resistant bacteria is rapidly increasing and eliminating treatment options for infected individuals. Every year in the United States, approximately 2 million people are infected with an antibiotic-resistant pathogen, and at least 23,000 people die as a result of the infection.[1,2] Early in 2017, the World Health Organization published a priority pathogens list of antibiotic-resistant bacteria on the basis of many factors, some of which included transmissibility and prevention in hospital or community settings.[3] Because of the overuse of antibiotics, there is a selective pressure in the environment for antibiotic resistance genes. The rise of antibiotic-resistant bacteria has led to a greater interest in infection control. Outbreaks of methicillin-resistant *Staphylococcus aureus* (MRSA) infections, primarily hospital-acquired infections, have been found in individuals involved in athletics.[4] Stanforth et al found that surfaces in high school wrestling facilities tested positive for community-acquired MRSA strains, putting athletes at a higher risk of MRSA infection.[4] In 2011, there were approximately 80,461 invasive MRSA infections. Of those infections, 60% were hospital-acquired infections, 17.5% were hospital-onset infections, and the remaining 22.5% were community-acquired infections (CAIs). Since 2005, the estimated infection rate for hospital-acquired infections dropped by 27.7%, hospital-onset infections dropped by 54.2%, and CAIs only dropped by 5%.[5] These findings demonstrate the need for proper hygiene protocols and other approaches to reduce the potential for CAIs in high-touch environments.

Copper and copper alloys are gaining increasing attention in infection control measurements because of their antimicrobial properties. The U.S. Environmental Protection Agency has

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recognized 5 families of copper alloys as antimicrobial, indicating that copper alloys reduce bacterial burdens by 99.9% within 2 hours of exposure.[6-12] Studies have found that copper surfaces rapidly reduce bacterial loads through a process known as contact killing against both gram-positive and gram-negative bacteria.[13-17] With the rise of antibiotic-resistant bacteria, such as MRSA, the use of copper alloy surfaces provides a mean of not only reducing bacterial burden, but also mitigating the spread of antibiotic-resistant bacteria in the environment. The implementation of copper alloys on high-touch surfaces in the hospital resulted in decreased bacterial counts on surfaces in comparison with control surfaces.[18-23] Souli et al found copper coating reduced bacterial burden associated high-touch hospital items and lowered the number of surfaces contaminated with multidrug-resistant bacteria.[24] The ability of copper to lessen bacterial loads rapidly is important for continuously touched surfaces and therefore can more readily reduce the transmission of antibiotic-resistant bacteria.

In the United States, 57.3 million people are members of at least one athletic facility.[25] Athletic facilities are becoming commonly frequented locations, with rates of gym visits expected to increase over the years. With the demonstrated success of copper alloys to significantly reduce bacterial counts in hospital settings, the aim of our study was to determine its efficacy in an athletic center setting and investigate the microbial communities on copper alloys placed on high-touch athletic center equipment. We hypothesized there would be a decrease in bacterial loads on copper alloy surfaces tested compared with bacterial loads found on standard noncopper equipment.

MATERIALS AND METHODS

Study site and cleaning regimen

The study was conducted at the Grinnell College Athletic Center, located in Grinnell, Iowa. The facility is regularly used by college athletic teams, students, faculty and staff of the college, and members of the local community. The cleaning protocol calls for student workers to wipe down machines, benches and bars with GymWipes Antibacterial Wipes (2XL Corporation, Forest Park, IL). Users of the athletic center are also encouraged to wipe down equipment with the GymWipes after their workout. The athletic center is cleaned every morning with Super HDQ L 10 (Spartan Chemical, Maumee, OH). Weights are not cleaned, and only attachments on equipment are wiped.

Study design and sample collection

This trial was designed to determine the bacterial burden on high-touch copper alloy and control athletic equipment over the course of 16 months in a college athletic center and characterize the most common bacteria found on the equipment. Five types of commonly used equipment and attachments made with copper alloy or control grips were tested, including dumbbells (sets at 6.8, 9.1, 11.3, 13.6, and 15.9 kg), barbells (13.6, 18.1, and 22.7 kg), kettlebells (6.8, 11.3, and 15.9 kg), specialty dumbbells (Triple Threat, 6.8 and 9.1 kg), grip attachments, lat pulldown attachments, and low row attachments. The grip areas of the copper alloy equipment were made from C706 copper alloy (90% copper, 10% nickel by weight), whereas the control equipment was rubber coated or exposed stainless steel. The copper alloy equipment and control equipment were rotated in the facility approximately every 3 weeks, and samples were collected 3 times per week. For the kettlebells, barbells, and Triple Threats (Black Iron Strength, Vancouver, WA), only the copper alloy equipment or control was available during the testing time. For the grips and dumbbells, copper alloy pieces were switched with controls; however, other control grips or weights were available, whereas copper alloy equipment was out because of the demand for >1 grip or set of weights.

Sampling was conducted as previously described by Attaway et al, with the following modification: 4- × 1-in Kimtech W5 wipes (Kimberly-Clark, Roswell, GA) premoistened with 400 µL of phosphate-buffered saline with 0.5% Tween 80 and 0.07% lecithin (PBS-LT) were used to allow for sampling around the grip.[26] Wipe samples were collected during open times at the athletic center or directly after it closed after morning hours during the summer. After sampling, each wipe was placed into 6 mL of PBS-LT buffer. Samples were vortexed, diluted with PBS-LT buffer, and plated onto trypticase soy agar with 5% sheep blood (TSAII; BD, Sparks, MD), with incubation for 48 hours at 37°C.

Bacterial characterization and antimicrobial susceptibility test

The most common bacterial morphologies on each plate were selected for identification. Bacterial DNA was isolated and 16S ribosomal RNA amplified as previously described.[27] Samples were sequenced at the University of Chicago Comprehensive Cancer Center DNA Sequencing and Genotyping Facility. Sequences were run through DECIPHER[28] to check for chimeras and Classifier (Ribosomal Database Project)[29] to assign genus. Sequences were deposited in GenBank (accession no. MF385203- MF385271 for copper alloy isolates and MF375118-MF375202 for isolates from control surfaces).

Antimicrobial susceptibility testing was carried out on *Staphylococcus* isolates following published protocols.[30] Bacteria were grown on Mueller-Hinton plates (BD) and tested against erythromycin (15 µg), vancomycin (30 µg), and linezolid (30 µg) (BD).

MRSA prevalence

A total of 99 samples were collected during the study, with 49 samples from copper-alloy equipment and 50 samples from control equipment. Samples were collected in the same manner as the bacterial burden protocol, and then plated on CHROMagar MRSAII (BD) following the manufacturer's instructions.

Statistical analysis

For each item, surface areas of sampled regions were measured and the colony forming units (CFU) per 100 cm^2 were calculated. Bacterial loads on copper alloy items versus noncopper items using nonparametric Mann-Whitney *U* test with a significance level at $P < .05$ using Prism 6 software (GraphPad Software, La Jolla, CA) were calculated.

RESULTS

Bacterial burden was significantly decreased on high-touch copper alloy surfaces compared with controls

Copper grips had significantly lower bacterial loads than control items, with an average decrease of 94% (n = 543 for copper and n = 536 for control). For every type of grip tested, reductions in bacterial counts decreased from 85%-97% on copper alloy surfaces (Table 1). Control dumbbells carried the highest bacterial loads, with kettlebells and specialty dumbbells carrying the lowest. The highest bacterial loads were found on barbells and specialty dumbbells for the copper alloy grips tested (Table 1). Control 15-lb dumbbells carried the highest bacteria counts with an average of 15,985 CFU/100 cm^2 (n = 34), whereas 40-lb barbells and 35-lb kettlebells carried the lowest (3,311 and 2,923 CFU/100 cm^2, respectively; n = 27 for barbell and n = 40 for kettlebell) (Fig 1). Copper grips had the highest bacterial counts on the 30-lb barbell with an average of 724 CFU/

Table 1
Bacterial burdens on equipment and attachment grips

Type of equipment	Copper components			Control components			P value	% reduction
	Mean CFU/100 cm²	Median CFU/100 cm²	n	Mean CFU/100 cm²	Median CFU/100 cm²	n		
Attachment	416	184	96	6,514	3,429	103	<.0001	94
Barbell	535	350	81	6,723	2,971	81	<.0001	92
Dumbbell	288	141	148	10,360	5,333	153	<.0001	97
Kettlebell	461	207	137	3,722	2,182	119	<.0001	88
Specialty dumbbells	551	331	81	3,777	2,524	80	<.0001	85

CFU, colony forming units.

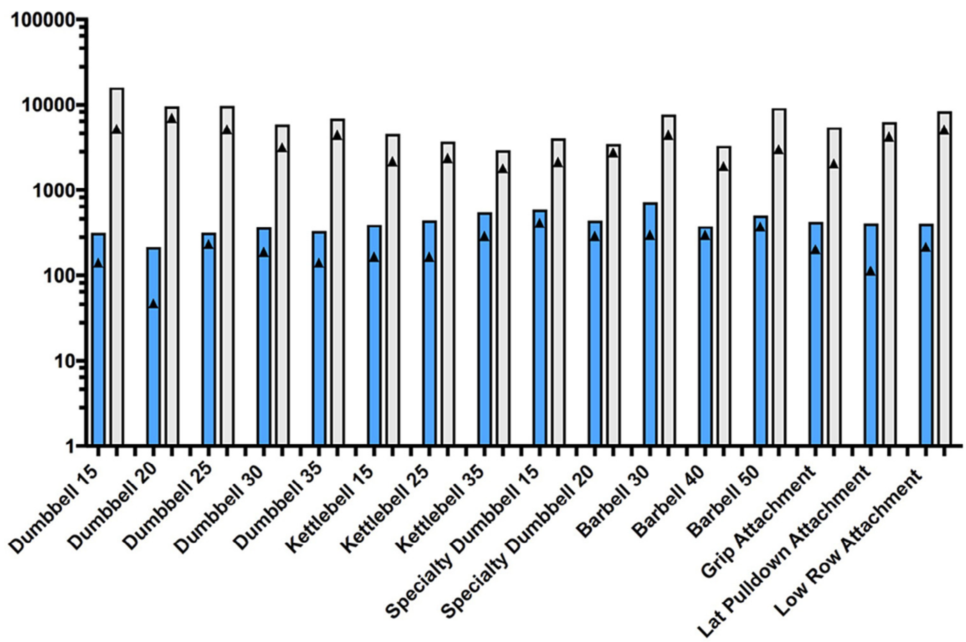

Fig 1. Copper alloy equipment reduced concentrations of bacteria on all equipment types. The mean bacterial concentrations recovered are shown by the bars, whereas the median concentrations are identified by the triangles. Copper alloy grips are represented by the blue bars and controls by the gray bars ($P < .0001$ for all pairs) (numbers range from 25-45 for individual items tested).

100 cm² (n = 27), whereas the lowest average was found on 20-lb dumbbells at 215 CFU/100 cm² (n = 33).

Characterization of the most common isolates found Staphylococcus *dominant in this setting and isolates showed low levels of resistance to 3 antibiotics tests*

Staphylococcus and *Micrococcus* were the 2 most prevalent bacterial genera found on both copper alloy and control grips, making up a combined 90% and 91%, respectively, of isolates characterized (Fig 2). Because *Staphylococcus* was most common genus of bacteria found on both types of surfaces, antibiotic susceptibility testing with vancomycin, erythromycin, and linezolid was conducted on 17 isolates from copper surfaces and 18 isolates from control surfaces. All isolates tested were sensitive to vancomycin and linezolid. Erythromycin resistance was found in 6 copper isolates (35%) and 8 control isolates (44%). Of the 99 samples tested for MRSA on CHROMagar MRSAII, no positive MRSA colonies were obtained.

DISCUSSION

In the United States, CAIs have persisted; therefore, maintaining cleanliness and hygiene in locations with high-touch surfaces, such as athletic centers, is important for reducing infection risks. This study examined the ability of copper alloys to facilitate in hygiene maintenance by controlling bacterial burden on athletic equipment grips. Our findings suggest that U.S. Environmental Protection Agency–registered copper alloys are able to reduce bacterial burdens by 94%, confirming previous observations on the antimicrobial properties of copper.[18,31,32]

Assessing the bacterial burden on equipment surfaces allows athletic centers to determine which pieces of equipment afford more attention in hygiene maintenance. Across all sampled equipment, the average concentration of bacteria recovered from surfaces was highest for control dumbbells, with a mean concentration of 10,360 CFU/100 cm² (Table 1). Dumbbells had the largest reduction in bacterial burden (97%), whereas specialty dumbbells showed the smallest reduction (85%). Nonetheless, for all equipment groups, copper alloy surfaces had significantly lower bacterial concentrations than control counterparts (Table 1). Although gym users are encouraged to disinfect equipment before and after usage, there is no guarantee that surfaces are being cleaned; therefore, equipment surfaces could go long periods of time without proper disinfection, harboring considerable loads of bacteria. The sustained antimicrobial properties of copper prove useful for continuous hygiene maintenance in locations that may not be regularly disinfected.

Our study also delineated the most common bacterial isolate morphologies associated with copper and control equipment surfaces.

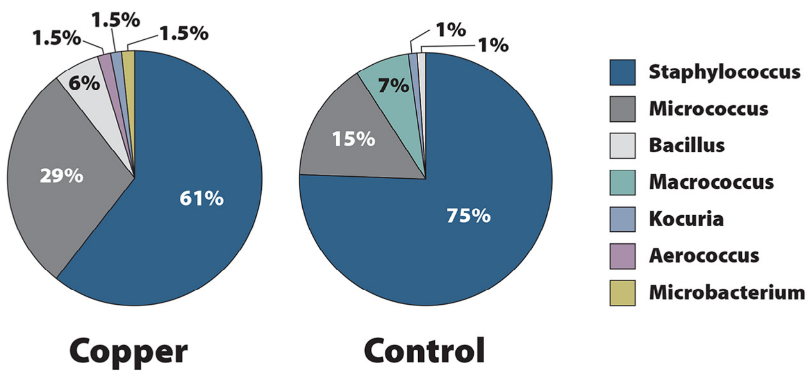

Fig 2. Characterization of the most common colony morphologies present on copper alloy and control surfaces. The most common morphologies from plates were isolated and the 16S ribosomal RNA genes were amplified and sequenced to determine the bacterial identify (n = 69 for copper and n = 89 for control).

The most common bacterial genus was found to be *Staphylococcus*. These findings support previous studies that have identified *Staphylococcus* as one of the most common bacteria found in indoor environments.[33-35] Bacteria from microbial communities of the built environment are associated with human skin, outdoor air, and soil microbiomes.[36-38] Investigating the bacterial diversity associated with gym equipment surfaces, Mukherjee et al determined the presence of *Staphylococcus*, with *Staphylococcus saprophyticus* being the most prevalent species, followed by *Staphylococcus epidermidis* and *S aureus*.[35] Each of these bacterial species can exhibit as opportunistic pathogens for humans[39-42] and can therefore pose a threat to individuals who frequent athletic facilities.

Because of the high prevalence of *Staphylococcus* and the risk of infection implicated to antibiotic-resistant species of *Staphylococcus*, antibiotic resistance susceptibility tests were conducted on a selection of *Staphylococcus* isolates collected. Resistance was only present against erythromycin. *Staphylococcus* spp have developed efficient mechanisms to evade the immune system and resist common antibiotics, such as penicillin, cefoxitin, and gentamicin.[43] The misuse of antibiotics at subinhibitory levels has been shown to promote bacterial colonization by stimulating enhanced biofilm formation.[44] The ability of *S aureus* to rapidly develop resistance to antibiotics presents a growing public health problem.[45] Antimicrobial copper has proved effective in killing pathogenic MRSA.[46,47] The use of copper could be a suitable solution to the rapid increase of antibiotic-resistant bacteria.

Community-associated MRSA has become a major concern for athletic facilities. Although extensive research on MRSA in hospital settings is available, there is a dearth of studies investigating the prevalence of MRSA on equipment surfaces in athletic facilities. Several reports of MRSA outbreaks in team sports and their training facilities highlight the importance of determining MRSA reservoirs in these environments and implementing cleaning protocols that most efficiently minimize risks of CAI.[4,48-51] Our study assessed the prevalence of MRSA in our testing site to determine if equipment surfaces could be a source of surface-to-skin contamination. We found no positive cultures of MRSA from either copper alloy or control equipment surfaces. Ryan et al found similar results, with no cultures positive for MRSA from 240 samples obtained from 5 gym surfaces.[52] Although our findings suggest that athletic centers may not be sources of community-acquired MRSA, our limited sample size of 99 cultures could be insufficient to capture the prevalence of MRSA colonization. Stiefel et al found that hospital surfaces, such as bed rails, bedside tables, and call buttons, served as vectors of surface-to-skin contamination of MRSA for both health care providers and patients.[53] These findings suggest that contamination of high-touch environmental surfaces could be sources of infection. Niiyama et al determined there to be significantly lower MRSA counts on bedsheets with metallic copper imbedded in them, preventing the spread of MRSA in dermatology wards.[54] Implementation of infection control strategies, such as the use of copper alloys in combination with disinfecting procedures, should be considered to augment cleaning protocols and mitigate the spread of CAIs.

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