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Is it Possible to Sanitize Athletes' Shoes?

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Context: Footwear should be designed to avoid trauma and injury to the skin of the feet that can favor bacterial and fungal infections. Procedures and substances for sanitizing the interior of shoes are uncommon but are important aspects of primary prevention against foot infections and unpleasant odor.

Objective: To evaluate the efficacy of a sanitizing technique for reducing bacterial and fungal contamination of footwear.

Design: Crossover study.

Setting: Mens Sana basketball team.

Patients or Other Participants: Twenty-seven male athletes and 4 coaches (62 shoes).

Intervention(s): The experimental protocol required a first sample (swab), 1/shoe, at time 0 from inside the shoes of all athletes before the sanitizing technique began and a second sample at time 1, after about 4 weeks, April 2012 to May 2012, of daily use of the sanitizing technique.

Main Outcome Measure(s): The differences before and after use of the sanitizing technique for total bacterial count at 36°C and 22°C for *Staphylococcus* spp, yeasts, molds, *Enterococcus* spp, *Pseudomonas* spp, *Escherichia coli*, and total coliform bacteria were evaluated.

Results: Before use of the sanitizing technique, the total bacterial counts at 36°C and 22°C and for *Staphylococcus* spp were greater by a factor of 5.8 (95% confidence interval [CI] = 3.42, 9.84), 5.84 (95% CI = 3.45, 9.78), and 4.78 (95% CI = 2.84, 8.03), respectively. All the other comparisons showed a reduction in microbial loads, whereas *E coli* and coliforms were no longer detected. No statistically significant decrease in yeasts ($P = .0841$) or molds ($P = .6913$) was recorded probably because of low contamination.

Conclusions: The sanitizing technique significantly reduced the bacterial presence in athletes' shoes.

Key Words: athlete's foot, foot infections, bacterial infections, fungal infections, basketball, hygiene

Key Points

- Microbes and pathogens can proliferate in athletes' shoes.
- Little research has been conducted on methods for sanitizing shoe interiors.
- The sanitizing technique was effective in reducing the bacterial load.

It is essential that we take care of our feet. They sustain our weight when we stand or move, adapting to any position we take. In a lifetime, a person could cover, on his or her feet, a distance equal to more than 3 times the Earth's circumference.[1]

Feet may be compromised by bacterial and fungal infections,[2–10] chronic disease,[10–13] obesity,[14] immune suppression,[9,10,15] vascular disease,[10,15] and uncomfortable or tight shoes. Tight shoes can injure the feet and make them prone to contamination and infections. Unventilated shoes are prone to bacterial and fungal proliferation.[16,17] Sweat is a nutrient for bacteria, and bacterial metabolism gives feet, socks, and shoes a strong odor.[18] Bacterial and fungal infections and proliferation are influenced by microclimate, temperature, humidity,[8] activity,[2,8,9,19] lifestyle,[20,21] and individual predisposition. Feet have a rich bacterial flora, most of which is not normally pathogenic if the feet are in good health. Lifestyle factors can expose the feet to higher risks of contamination by certain bacteria. For example, going barefoot exposes feet to contamination by *Escherichia coli* and other potential pathogens. Patients with circulatory problems and certain chronic conditions are susceptible to infection by *Staphylococcus aureus, Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and the coliform group of bacteria[3,13] (especially *Enterococcus* spp). Sport centers are notorious sources of onychomycotic infections.[6,8,22]

Good hygiene is certainly the first step for healthy feet but is not always sufficient. Feet and socks are easily washed and disinfected, whereas the insides of shoes are often neglected. New methods and products that effectively sanitize the insides of shoes are an important aspect of primary prevention against foot infections and strong odor.[18,23] For this study, we evaluated the effectiveness of a sanitizing technique in the form of putty, and we measured shoe contamination levels before and after application of the product.

METHODS

Settings

The crossover study was conducted from April 2012 to May 2012 and involved the 2012 Mens Sana basketball

team (Siena, Italy). Before explaining the study to the athletes, we arranged a meeting among the principal researcher (G.M.), the medical staff of Mens Sana, and the Mens Sana coaches to describe the project, establish contacts, and schedule meetings between athletes and university staff. The project was presented 3 times to include all the athletes involved in the study; so as not to miss athletes absent at the first encounters, we also organized 2 ad hoc meetings. We informed the athletes about the research and provided material and photographs of culture plates inoculated with swabs from treated and untreated shoes. Visual feedback was calculated to motivate athletes to follow the instructions on proper use of the product. We asked the athletes to wear the same shoes for the entire duration of the study, and if that was not possible, to report otherwise. Several cohorts were followed because the athletes did not all train or play the sport together. Thus, several meetings were arranged each week to replace the product.

Study Population

We invited 34 athletes to take part in the project: 1 athlete (3%) refused, giving no reason, and 33 (97%) accepted. Two of the 33 (6%) were subsequently excluded because of the irregularity of their weekly training. The final study population was 31 athletes (1 was in the under-16-years group [3%], 15 were in the under-17-years group [48%], 11 were in the under-19-years group [35%], and 4 [13%] were male coaches). The final number of shoes examined was 62.

We obtained informed consent from the athletes or their parents and followed the principles of the Declaration of Helsinki. The under-16-year-old athlete wore the shoes for about 2 h/d, 3 times/wk. Athletes in the under-17 and under-19 groups trained 5 to 6 times/wk, wearing the shoes 2 h/d. The coaches wore the shoes 6–7 d/wk for more than 8 h/d.

We verified the hygiene habits of the athletes, emphasizing that they should bring their shoes to training in their bags, wear flip-flops when they were in the shower or locker room, and change their socks before they start exercising.

Disinfecting Technique

We used a putty compound, Cyberclean for shoes (Joker Group, Kerzers, Switzerland), which has a malleable and elastic consistency, adhering to and removing dirt. It consists mainly of the natural ingredient guar and ethanol with colorants, odorants, and clotrimazole, an antifungal agent.

Data Collection

Research fellows of the Department of Molecular and Developmental Medicine were charged with collecting samples. The experimental protocol required a first sample (swab), 1/shoe, at time 0 (T_0) from inside the shoes of all athletes before use of the product began and a second sample at time 1 (T_1), after about 4 weeks of daily use of the product. The first samples were taken immediately after presentation of the project; this was important because participants could have been prompted to modify hygiene for the study. Participants were supplied with the product and instructions on how to use it. In particular, those instructions recommended that participants use the product on dry shoes before wearing them and insert the product into the interior of the shoe with pressure for about 10 seconds, without rubbing. To cover the whole interior of each shoe, that operation could be repeated 2 to 3 times, and then the product was to be replaced in its original box. Participants were instructed to use the product before training sessions and matches. The principal researcher (G.M.) collected the product each week and provided athletes with new packs. If athletes were absent at the time of replacement, the new product was given to the coaches to pass on to the athlete in exchange for the old pack at the first possible occasion. Product distribution and withdrawal was traced by user identification. The same tracking procedure was also used by the operator who collected the shoe swabs and seeded and read the plates at T_0 and T_1. We asked the athletes to indicate whether they used other products, such as antifungal or antibacterial agents or changed their hygiene practices during the study.

During the study period, 126 product packs were provided to participants; 19 packs (15%) were lost or not returned. Twelve meetings were arranged to substitute old products with fresh during the study. The second sampling, T_1, was conducted on 4 different dates.

All the data, including results of the laboratory analyses, were stored in a database. The data were cleaned to remove incomplete, incorrect, or inaccurate items before analysis.

Laboratory Analysis

Culturing of the swabs was carried out in the Hygiene and Environmental Laboratory of the University of Siena (Department of Molecular and Developmental Medicine). Because of the variety and quantity of bacteria found in the shoes, the first culture consisted of overall bacteria counts at 36°C and 22°C on nonselective plate count agar. That medium enables culture of mesophilic bacteria at 36°C, reflecting atopic contamination, and of psychrophilic bacteria at 22°C, reflecting environmental contamination.

Samples were then cultured for specific bacteria to highlight the following: (1) fecal contamination by *E coli* and *Enterococcus* spp and (2) ubiquitous species, such as *Pseudomonas* spp and *Staphylococcus* spp, which are potentially pathogenic. The presence of molds and yeasts was investigated by culturing on Sabouraud dextrose agar medium at 22°C.

Samples were obtained by swabbing the inner surface of the shoe sole and upper parts with sterile pads. In the laboratory, the swabs were placed in phosphate-buffered saline and shaken in a vortex mixer; the liquid was seeded (0.1 mL/plate) in several petri dishes containing plate count agar (PCA) for the total microbial loads of mesophilic and psychrophilic microorganisms incubating at 36°C and 22°C, respectively; Sabouraud dextrose agar for yeasts and molds incubating at 22°C; mannitol salt agar for *Staphylococcus* spp; *Pseudomonas* Cetrimide for *Pseudomonas* spp; Slanetz and Bartley medium for *Enterococcus* spp; and selective chromogenic medium for *E coli* and bacteria in the coliform group, incubating at 36°C. The results were expressed as colony-forming units (CFUs) per plate. The plates were read 24 and 48 hours after seeding, except for yeasts and molds, which were read at 72 hours.

Table 1. Comparison of Total Bacterial Count in Shoes Before and After Sanitizing for the 62-Shoe and 46-Shoe Studies

		All Shoes (n = 62)						Some Shoes Excluded (n = 46)					
Variable	Temperature[a] and Time	Total Count[a]	Mean ± SD	Median	Interquartile Range	Minimum	Maximum	Total Count[b]	Mean ± SD	Median	Interquartile Range	Minimum	Maximum
Plate count agar													
	36°C at T_0	11 766	189.77 ± 466.38	32.5	12–81	3	2400	4577	99.5 ± 157.50	39	18–81	5	719
	36°C at T_1	757	12.21 ± 14.74	7	3–14	1	88	664	14.43 ± 16.27	9.5	4–18	1	88
	22°C at T_0	13 428	216.58 ± 574.94	45	14–115	2	3000	4201	91.33 ± 126.21	45	18–86	2	589
	22°C at T_1	867	13.98 ± 19.42	8	4–17	0	125	780	16.96 ± 21.65	9.5	4–22	0	125
Staphylococcus spp	T_0	8050	129.84 ± 231.78	29	14–78	1	1036	4760	103.48 ± 159.7	33	17–78	2	664
Staphylococcus spp	T_1	1064	17.16 ± 25.17	7.5	3–23	0	152	986	21.43 ± 27.87	11	4–28	0	152
Yeast	T_0	1960	31.61 ± 146.95	0	0–3	0	822	292	6.35 ± 23.91	0	0–3	0	152
	T_1	272	4.39 ± 26.64	0	0–1	0	210	266	5.78 ± 30.89	0	0–1	0	210
Mold	T_0	3	0.05 ± 0.22	0	0	0	1	3	0.07 ± 0.25	0	0	0	1
	T_1	5	0.08 ± 0.33	0	0	0	2	5	0.11 ± 0.38	0	0	0	2
Pseudomonas	T_0	0	NA	NA	NA	0	0	0	NA	NA	NA	0	0
	T_1	0	NA	NA	NA	0	0	0	NA	NA	NA	0	0
Enterococci	T_0	661	10.66 ± 37.51	0	0–2	0	217	147	3.20 ± 10.16	0	0–1	0	64
	T_1	37	0.6 ± 3.7	0	0	0	29	36	0.78 ± 4.29	0	0	0	29
Escherichia coli	T_0	232	3.74 ± 18.08	0	0	0	130	3	0.07 ± 0.33	0	0	0	2
	T_1	0	NA	NA	NA	0	0	0	NA	NA	NA	0	0
Total coliform	T_0	526	8.48 ± 40.53	0	0	0	298	32	0.7 ± 3.54	0	0	0	24
Total coliform	T_1	0	NA	NA	NA	0	0	0	NA	NA	NA	0	0

Abbreviations: NA, not applicable; T_0, before sanitizing; T_1, after sanitizing for 1 month.

[a] Where applicable.

[b] Colony-forming units/plate with 0.1 mL.

Table 2. Bacterial Loads Before and After Sanitizing Shoes for the 62-Shoe and 46-Shoe[a] Studies

Sample	Bacteria	Geometric Mean at T_0 and T_1				Ratio of Geometric Means		
		T_0	95% CI	T_1	95% CI	T_0/T_1	95% CI	*P* Value
62 shoes	Plate count agar at 36°C	40.54	26.78, 61.37	6.98	5.3, 9.21	5.8	3.42, 9.84	<.001
	Plate count agar at 22°C	45.76	29.88, 70.07	7.83	5.76, 10.64	5.84	3.46, 9.87	<.001
	Staphylococci	38.79	25.49, 59.04	8.12	5.72, 11.53	4.78	2.84, 8.03	<.001
46 shoes	Plate count agar at 36°C	41.81	28.47, 61.40	8.39	6.06, 11.64	4.98	2.99, 8.29	<.001
	Plate count agar at 22°C	41.16	27.74, 61.05	10.19	7.10, 14.64	4.04	2.41, 6.78	<.001
	Staphylococci	40.8	26.68, 62.39	11.38	7.75, 16.70	3.59	2.09, 6.16	<.001

Abbreviations: CI, confidence interval; T_0, before sanitizing; T_1, after sanitizing for 1 month.

[a] Using paired *t* tests.

Statistical Analysis

Descriptive analysis of the data for all types of microbes and molds was performed at T_0 and T_1 (Table 1). To reveal differences in bacterial contamination before and after use of the product, parametric (*t* test for paired data) and nonparametric (Wilcoxon signed rank) tests were used. The parametric approach required data transformation to meet the normality requirement (differences are required to have a normal distribution, and standard deviations had to be constant across the range of differences before and after use of the product). The following types of transformation were tried on the raw data: cubic, square, identity, square root, logarithmic, 1/square root, inverse, 1/square, and 1/cubic. Besides normality testing, we used graphic (descriptive plots: stem-and-leaf plot, dot plot, box plots, histograms, P plots, and Q-Q plots) and numerical (Shapiro-Wilk test, Jarque-Bera test, and skewness-kurtosis test) approaches. The logarithmic transformation function best satisfied the paired *t* test assumptions for PCA at 36°C and 22°C and *Staphylococcus* spp. Transformed data were analyzed using the paired *t* test. The raw data were transformed before calculating the differences, and in this case, the assumption was that the differences after transformation had a normal distribution. Differences and confidence intervals were back transformed to natural scale. Back-transformed means are no longer arithmetic but become geometric, which allow comparison with ratios.[24]

Besides the paired *t* test used for PCA at 36°C and 22°C and *Staphylococcus* spp, the Wilcoxon signed rank test was also used to determine any statistically significant differences in CFUs between T_0 and T_1. Yeasts, molds, *E coli*, coliform bacteria, and *Enterococcus* spp were studied using the Wilcoxon signed rank test because it was not possible to use the paired *t* test. The following comparisons and analyses were conducted:

1. We measured shoe contamination before use of the product in the different age groups of athletes to determine whether they had the same level of contamination; we excluded the under-16-year-old group because it contained only 1 participant.
2. We performed a quantitative total count of CFUs from all participants (62 shoes) to measure the overall effect of product use on the different types of microorganisms and molds considered, as well as a count indicating the presence or absence of CFUs between T_0 and T_1.
3. We conducted further data analysis, removing possible outliers and possible irregular shoe use, and running the analysis on a subgroup of 46 shoes (74%).
4. We did a further analysis on coaches' shoes because the coaches wore their shoes about 8 h/d, compared with only about 2 h/d for the athletes.

Stata SE software (version 12.1; StataCorp, College Station, TX) was used for the analysis. Significance was set at $P < .05$.

RESULTS

The CFUs of samples at T_0 and T_1, total overall CFU count, means, standard deviations, minima, maxima, medians, and interquartile ranges are presented in Table 1.

The following reductions in CFUs were recorded in both the 62-shoe and 46-shoe groups at T_1: 85.5%–93.6% for PCA at 36°C; 81.4%–93.5% for PCA at 22°C; 79.1%–86.8% for *Staphylococcus* spp; 8.9%–86.1% for yeasts; 75.5%–94.4% for *Enterococcus* spp; 100% for *E coli* and coliform group. No sample contained *Pseudomonas* at T_0 or T_1. Total molds detected in the samples increased from 3 at T_0 to 5 at T_1. Shoe contamination (number of positive shoes out of 62) decreased: 4.8% for PCA at 22°C and *Staphylococcus* spp from 62/62 at T_0 to 59/62 at T_1; 72.7% for *Enterococcus* spp, from 22/62 at T_0 to 6/62 at T_1; 100% for *E coli* and coliform group, 6/62 and 13/62 to 0 for both of them.

The paired *t* test applied to PCA at 36°C, PCA at 22°C and *Staphylococcus* spp showed reductions in both the 62-shoe and 46-shoe samples. In particular, when all 62 shoes were analyzed with PCA at 36°C, the number of colonies was greater by a factor of 5.80 (95% confidence interval [CI] = 3.42, 9.84) before use of the product at T_0 ($P < .001$); the corresponding factor was 5.84 (95% CI = 3.46, 9.87) for PCA at 22°C ($P < .001$) and 4.78 (95% CI = 2.84, 8.03) for *Staphylococcus* spp ($P < .001$). When we conducted the same analysis on the 46-shoe sample, the number of colonies developing on PCA at 36°C was greater by a factor of 4.98 (95% CI = 2.99, 8.29) before use of the product at T_0 (P < .001); the corresponding factor was 4.04 (95% CI = 2.41, 6.78) for PCA at 22°C ($P < .001$) and 3.59 (95% CI = 2.09, 6.16) for *Staphylococcus* spp ($P < .001$) (Table 2).

The Wilcoxon signed rank test highlighted a reduction ($P < .001$) in *Enterococcus* spp in the 62-shoe and 46-shoe samples. No statistical test was applied to differences in the *E coli* and coliform group because at T_1, none of the shoes were contaminated by these microbes. No change was found for molds ($P = .6913$ and $P = .6861$) or yeasts ($P = .0841$ and $P = .1956$) in the 62- and 46-shoe samples, respectively, although this was probably due to the low level of initial mold and yeast contamination of shoes.

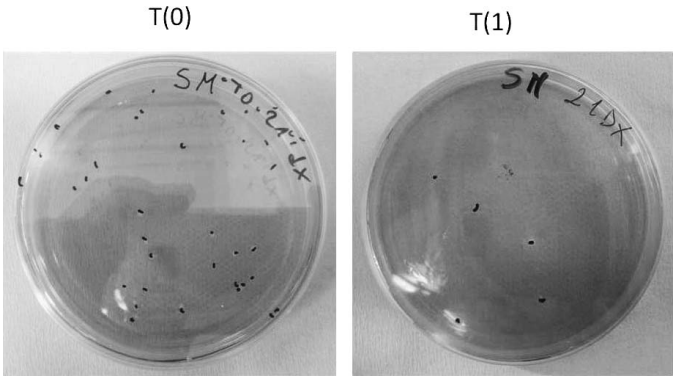

Figure 1. Petri dishes containing mannitol salt agar for *Staphylococcus* spp. Abbreviations: T(0), before sanitizing; T(1), after sanitizing for 1 month.

The Wilcoxon signed rank test applied to the results for the 8 shoes worn by coaches at T_0 and T_1 highlighted the borderline statistical significance ($P = .0663$) of a 67.8% reduction in overall bacterial load between T_0 and T_1 for PCA at 36°C; the corresponding results for PCA at 22°C were borderline significant ($P = .0506$) for a 72.4% reduction and significant for *Staphylococci* ($P = .0382$) with an 82.2% reduction in overall bacterial load. No statistical difference ($P = 1.802$) was found for yeasts between T_0 and T_1. The sample (8 shoes) was too small to obtain reliable results for the coaches' group. A larger sample would probably have revealed marked differences.

We did not find any statistically significant difference in the microbial load of shoes before using the product in the different groups of athletes: under 17 years, under 19 years, and coaches ($P > .05$).

Examples of petri dishes showing differences in CFUs at T_0 and T_1 are displayed in Figures 1–3.

DISCUSSION

Onychomycosis, tinea pedis, and bacterial foot infections have a high prevalence in the general population.[5,10] Although these diseases are not important in terms of mortality, their effects may lead many patients to develop psychological, physical, and social impairments, with reduced quality of life and limitation of interaction with others.[9–15]

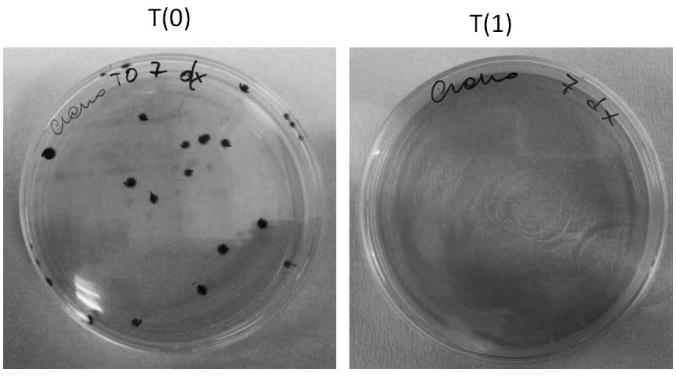

Figure 2. Petri dishes containing selective *Escherichia coli* coliform chromogenic medium for *E coli* and coliform bacteria. Abbreviations: T(0), before sanitizing; T(1), after sanitizing for 1 month.

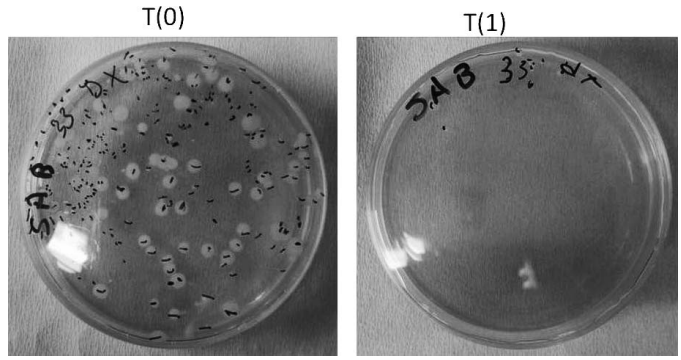

Figure 3. Petri dishes containing Sabouraud dextrose agar for yeasts and molds, incubated at 22°C. Abbreviations: T(0), before sanitizing; T(1), after sanitizing for 1 month.

People who actively practice sports are more susceptible to bacterial and fungal infections.[9] Indeed, epidemiologic studies reveal that tinea pedis (so-called athlete's foot) is closely associated with sports and is one of the most common dermatophytoses in sport-active individuals.[21] For example, Pickup and Adams[25] reported that the prevalence of tinea pedis in professional soccer players was higher than in nonathletic men. Bolanos[26] noted a higher incidence of tinea pedis in individuals participating in a swimming course on day 12 of the course (22%) than on day 1 (13%). In contrast, Purim et al[8] reported no differences in the prevalence of tinea pedis or onychomycosis among Chinese and Brazilian soccer players compared with nonathletes. Few studies in the literature deal with bacterial foot infections, particularly in athletes, despite their potentially negative consequences. An infected fissure caused by athlete's foot is often a portal for pathogens and can lead to cellulitis of the lower limb.[27] Little research has been done on shoes as a possible source of microbial contamination, despite the likelihood that shoes are rarely completely clean inside. Contamination is not always sufficient to determine an infection but remains a possible source, especially if the feet have lesions. Most authors[2,8,28] analyze feet, whereas we studied shoes.

Our results showed various microbes, some possibly involved in foot infections, in basketball players' shoes. We found a high presence of *Staphylococcus* spp, followed by yeasts, *Enterococcus* spp, the coliform group (including *E coli*), and molds.

At time T_0, before use of the product, the following total CFUs/plate counts were recorded in the 62-shoe sample: 8050 for *Staphylococcus* spp, 1960 for yeasts, 661 for *Enterococcus* spp, 526 for total coliform bacteria (including 232 CFUs for *E coli*), and 3 for molds. No CFUs were recorded for *Pseudomonas* spp. With the aim of reducing possible outliers, we reanalyzed the data set, excluding 16 shoes that could have been biased by certain athletes' behaviors. In the remaining 46 shoes, the CFU/plate findings were similar: 4760 for *Staphylococcus* spp, 292 for yeasts, 147 for *Enterococcus* spp, 32 for the coliform group (including 3 for *E coli*), and 3 for molds.

Similarly, Li et al[29] isolated 13 strains of bacteria from 12 pairs of children's shoes. They identified *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Staphylococcus aureus*, *Bacillus thuringiensis*, and *Bacillus* spp (strains CO64, DC3158, LY). *Staphylococcus aureus*,

an aggressively infectious agent, also produces toxins and may severely damage the skin.[30]

As are children, athletes are usually engaged in many physical activities and are, therefore, at high risk of severe foot diseases.[29] Other investigators[6] recorded a high prevalence of *Staphylococcus aureus* contamination in 2 sport teams (footballers and wrestlers). The authors isolated bacteria from the fingertips, knuckles, forearms, nostrils, and shoe soles of athletes and a control group. *Staphylococcus aureus* was more frequent in athletes.[6]

Many athletes neglect hygiene, despite being exposed to risk factors such as common showers, locker rooms, and saunas; swimming pool floors; synthetic fabrics; and unventilated shoes. Athletes are also susceptible to minor foot trauma, which favors fungal growth.[27]

More emphasis on measures for infection control, such as health, hygiene, foot care, and shoe-care education is, therefore, warranted. Patients with chronic illnesses, such as diabetes and vascular diseases, require special precautions for their feet, including regular foot examinations by a physician or podiatrist and daily, visual self-inspection.[1]

Shoe cleanliness is a substantial part of foot hygiene and is, therefore, of primary importance in preventing foot infections. In evaluating the effectiveness of the shoe-sanitizing product, we noticed a general reduction in the microbial population after use. That effectiveness was probably also due to the elastic consistency that allowed it to adapt to various surfaces and to remove dirt. In the 62-shoe sample, we recorded a reduction of more than 86% in CFUs of all microorganisms except molds. In the 46-shoe sample, we recorded a reduction of more than 75.5% in CFUs for all microorganisms. A significant borderline reduction ($P = .0841$) was recorded for yeasts (−8.9%). In both samples, molds increased from 3 to 5 CFUs/plate. The apparent ineffectiveness of this product for combating molds was probably due to the relative, initial absence of molds in the shoes. To test the product for molds, greater initial mold contamination of the shoes would be necessary. It would also be interesting to test whether the product inactivates quiescent fungal spores.

Cyberclean was effective and could be useful in a variety of situations, including children's shoes and in those engaged in physical activity. Many athletes practice a high level of foot care, including pedicures and frequent changes of socks and shoes,[9] because serious foot infections could mean missing sporting events and competitions, causing economic damage for athletes, sports clubs, and teams. Primary prevention may avoid foot infections and reduce athlete health costs. The benefits could be tested on specific populations, such as people with chronic conditions, such as diabetes, vascular diseases, and structural defects of the foot. Aerobic Gram-positive cocci (*Staphylococcus aureus* and β-hemolytic streptococci) and aerobic Gram-negative bacilli (*E coli*, *Proteus*, *Klebsiella*, and *Pseudomonas* spp) are often responsible for diabetic foot infections, which are a frequent cause of lower extremity amputation. A similar study could be conducted in a diabetic population to verify whether improved shoe hygiene was associated with better outcomes.

Cyberclean was presumably also effective against shoe odor, which is mistakenly believed to be caused by sweat but is actually due to bacteria within the shoes.[18,29] Ara et al[18] analyzed foot-odor components with sensory tests, isolated microorganisms that produced the odors, and evaluated the odors' causal mechanisms. Foot odor was derived from isovaleric acid, produced by the breakdown of leucine in sweat by *Staphylococcus epidermidis*, a normal component of cutaneous microbial flora. *Bacillus subtilis* was also detected in the plantar skin of participants with strong foot odor.[18] Thus, the product reduced shoe odor by reducing the number of bacteria. Participant perception of bad odor after use of the product could be investigated.

We evaluated precleaning contamination among the 3 groups (under 17 years old, under 19 years old, and coaches) without finding any differences among the groups, which means that the groups had similar levels of contamination.

We endeavored to prevent and to detect any potential causes of bias. When we discovered possible causes for bias, such as irregular use of the product, we excluded those cases and reanalyzed a subgroup of 46 of the original 62 shoes (74%). The results did not change.

Possible study limitations included conditions that we were unable to test for. We did a 1-month follow-up, but extended observations and testing of the shoes several times would be interesting. Resources and time did not allow that. It would be interesting to know whether the product can keep microbe concentrations low during a longer period. The shoes tested were in good condition. Professional athletes have high annual shoe-turnover rates, which can influence contamination levels; that is, higher bacterial loads can be expected in shoes used for longer periods. Repeating the study on a population whose shoes are used for years would be instructive. Finally, Cyberclean was used intensively (before every training session). If the product was used less rigorously or stored for long periods, instead of a fresh supply being provided, the results might have been different.

Evaluating the relationships between shoe cleaning and foot infections (that is, relating use of the product to a reduction in infections) would be helpful but would require a longer follow-up period and a larger sample group for statistical purposes.

Our results suggest that the disinfecting technique was effective in reducing shoe microbiologic load and was, therefore, useful for primary prevention of bacterial load in participants engaging in physical activity and could possibly be of use in patients with chronic diseases or foot deformities. The product's apparent ineffectiveness against molds suggests the need for further testing in a larger population with a greater prevalence of molds because we observed an initial, relative absence of mold in the athletes' shoes.

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