PennState Extension

Survival of Staphylococcus aureus on Synthetic Turf

Staphylococcus aureus is a common bacterium, but is capable of causing diseases ranging from minor soft tissue infections and food poisoning to serious medical problems such as toxic shock syndrome.

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Introduction

Staphylococcus aureus is a common bacterium found on human skin and in the nasal cavity. Typically the presence of the bacterium causes no problems and goes unnoticed. Occasionally, *S. aureus* is capable of causing diseases ranging from minor soft tissue infections and food poisoning to serious medical problems such as toxic shock syndrome. (Marples et al, 1990; Bennet and Lancette, 1998).

Most *S. aureus* infections are believed to be transmitted either via person-to-person contact or through a common source or shared items. Shaving and other activities which create breaks in the skin or abrasions have also been implicated in the spread of *S. aureus* among athletes (Begier et al, 2004). In a study examining the survival of bacteria on various synthetic surfaces including polyethylene, *S. aureus* was able to survive for relatively long periods of time (22 to 40 days) under ideal conditions (Neely and Maley, 2000).

Recently, outbreaks of antibiotic resistant strains of *S. aureus* have gained media attention. These outbreaks have resulted in the temporary closing of school buildings and athletic facilities while the facilities are cleaned. Strains of *S. aureus* resistant to antibiotics were first discovered in the 1960s, but until recently were mostly limited to hospital settings (Panlilio et al, 1992).

Methicillin resistant *S. aureus* (MRSA) isolates have become more prevalent among athletes (Kazakova et al, 2005). While there has been concern that infilled synthetic turf systems play a role in staph infections in athletes, conclusive evidence is not currently available.

The objective of this study is to examine the survival of *S. aureus* on infilled synthetic turf systems and natural turfgrass under different environmental conditions and to evaluate the effectiveness of various control agents applied to the synthetic turf.

Materials & Methods

One indoor and three outdoor studies were performed.

Indoor Study

TREATMENTS

Individual experimental units were prepared by cutting the carpet backing containing the tufted upright polyethylene slit film pile to fit into 10 boxes. Each box was 190 by 300 mm in size. Ambient crumb rubber infill was added to the boxes and worked into the pile to a depth of 24-27 mm to simulate a synthetic infill system. The Kentucky bluegrass (*Poa pratensis*, L.) experimental unit was prepared approximately one year in advance by seeding Kentucky bluegrass onto a shallow box containing 5 cm of a USGA specified sand rootzone. The box was kept in a greenhouse.

All experimental units were inoculated with an isolate of *S. aureus* except the untreated control which received no bacteria. The isolate of *Staphylococcus aureus* subsp. *aureus* was obtained from American Type Culture Collection (ATCC). The bacterium was originally isolated from a human lesion and has no antibiotic resistance. Sufficient quantity of the inoculum was obtained by transferring the *S. aureus* culture to petri plates containing tryptic soy agar (TSA) and incubating the plates at 36°C (Bennet and Lancette, 1998). The bacterial suspension was produced by adding sterile distilled water to the TSA plates and dislodging the bacteria from the agar with a sterile loop. The bacterial suspension was then collected and the concentration was adjusted to 1 x 10^4 colony forming units (CFU)/ml.

Individual experimental units were inoculated by applying the bacterial suspension to the infilled surfaces or Kentucky bluegrass turfgrass using a sterile needleless syringe and then rolling the surface with a 10 cm foam paint roller to distribute the bacteria. Bacteria were applied at a rate so that there were approximately 156 CFU/cm^2 for the higher rate and approximately 16 CFU/cm^2 for the lower rate. All experimental units were inoculated at the higher rate except the treatment labeled low rate *S. aureus* which was inoculated at the rate of 16 CFU/cm^2.

A cationic surfactant, SportsClean, (Coatings Specialist Group, Rochester Hills, MI) was applied to two of the infilled surfaces at a rate of approximately 1.3 liters surfactant per square meter of surface. This was sufficient to wet the fibers and the uppermost layer of crumb rubber with the surfactant. As per label directions, the surface was wiped with a cloth after 3 minutes to further distribute the

surfactant and then allowed to dry. One experimental unit was treated with the surfactant one hour prior to inoculation with *S. aureus* (Surfactant 1 hr prior) and another experimental unit was treated 25 days prior to the inoculation.

An antimicrobial product, SportsAide (Coatings Specialist Group, Rochester Hills, MI) was applied to three of the infilled experimental units. The antimicrobial was diluted according to the label and applied at a rate of approximately 1.3 liters product per square meter of surface. The three experimental units were as follows. The first was applied to the same experimental unit receiving the surfactant 25 days before bacterial inoculation and is termed A+S 25 days prior. The antimicrobial was also applied alone to another experimental unit 25 days prior to inoculation and is termed Antimicrobial 25 days prior. A third experimental unit was treated with the antimicrobial alone 1 hr prior to inoculation and is termed Antimicrobial 1 hr prior. This treatment was allowed to dry prior to inoculation. The two experimental units treated 25 days prior to inoculation were kept undisturbed in a controlled indoor environment away from sunlight for 25 days before being inoculated with *S. aureus*.

On the day of inoculation with *S. aureus*, the surface of another experimental unit was treated with a liquid detergent (Tide) at the rate of 25 ml detergent per square meter (35 gal. per football field) 1.5 hours prior to inoculation (Detergent 1.5 hr prior). A different experimental unit was treated with the same rate of detergent 15 minutes after inoculation and lightly brushed (Detergent post). For both treatments the detergent was diluted by a 1 to 50 ratio with water before application.

Additional treatments included: an inoculated infill system with no applied detergent, surfactant, or antimicrobial (High rate *S. aureus*); an infill system with no applied detergent, surfactant, or antimicrobial inoculated at the rate of 16 CFU/cm^2 (Low rate *S. aureus*); Kentucky bluegrass sod inoculated at the 156 CFU/cm^2 (Kentucky bluegrass); and an untreated uninoculated control (untreated control).

The ten treatments are shown in Table 1.

Table 1. Treatments for indoor study in controlled environment.

Treatment
Untreated control
Low rate *S. aureus*
High rate *S. aureus*
Antimicrobial 1 hour prior
Surfactant 1 hour prior
Antimicrobial 25 days prior
A+S 25 days prior
Kentucky bluegrass
Detergent post
Detergent 1.5 hour prior

EVALUATION PROCEDURES

Experimental units were sampled at 1, 2, 3, and 4 hours after inoculation on Day 1 for all experimental units except the Untreated control, Low rate *S. aureus*, and High rate *S. aureus*. These three treatments were sampled at 1 and 4 hours after inoculation on Day 1. Thereafter, experimental units were sampled daily for a total of 12 days. Sampling was conducted in two ways. Petri plates (50 mm diameter) containing Baird-Parker (BP) agar were pressed onto the surfaces of each experimental unit. Different locations were selected for each sampling. Data reported is the average of three sub-samples taken for each sampling time at different locations for each experimental unit. While this method sampled both the fibers and the surface crumb rubber, the amount of crumb rubber being tested by this method was small. So in addition to the petri plate method, at each sampling time a crumb rubber sample was also taken from selected treatments using a sterile test tube, 5 ml sterile distilled water was added to the crumb rubber in the test tube and shaken. A 0.1 ml subsample of the liquid was plated onto 85 mm BP agar plates and spread using a sterile inoculating loop. All plates were parafilmed and incubated at 36C. After 48 hours, colonies on the plates were counted.

Outdoor Studies

TREATMENTS

Outdoor experiments were conducted in May, July, and August 2008. An existing 6 year old infilled synthetic surface ForeverGreen (Levittown, Pennsylvania) was used for the outdoor portions of this study. The pile fibers were slit-film polyethylene and the infill was 100% ambient crumb rubber. Individual treatment areas were 20 by 25 cm with a minimum 30 cm border between areas.

A treatment area of similar size was delineated on adjacent established Kentucky bluegrass turfgrass. This area was approximately 10 meters from the synthetic turf treatment areas.

All experimental units were inoculated using the method described for the indoor study with an isolate of *S. aureus* except the untreated control which received no bacteria. Bacteria were applied at a rate so that there were approximately 16 CFU/cm^2 for each treated area.

The cationic surfactant, SportsClean, described in the indoor study was applied to the treatment area at a rate of approximately 1.3 liters surfactant per square meter of surface 25 or 14 days prior to inoculation with bacteria. The rate of surfactant was sufficient to wet the fibers and the uppermost layer of crumb rubber. As per label directions, the surface was wiped with a cloth after 3 minutes to further distribute the surfactant and then allowed to dry. The antimicrobial product, Aegis AEM 5772/4.2 (Pioneer Athletics, Cleveland, OH), was then applied to the same treatment area receiving the surfactant. The Aegis product was diluted according to the label and applied at a rate of approximately 1.3 liters product per square meter of surface. This treatment is termed: Antimicrobial + surfactant in the three outdoor studies.

On the day prior to inoculation with *S. aureus*, the surface of another treatment area was treated with a liquid detergent (Tide) at the rate of 5.7 ml detergent per square meter (8 gal. per football field). This treatment is labeled 'Detergent 1 day prior' in all three outdoor studies. This liquid detergent is an anionic surfactant. Three additional liquid detergent treatments were included in all the outdoor studies. Detergent was applied 1.5 hours prior to inoculation (Detergent 1.5 hr prior) at two rates, 5.7 ml detergent per square meter and 14.3 ml per square meter (20 gal. per football field). A third treatment consisted of applying the detergent at the 5.7 ml per meter squared rate 15 minutes after inoculation.

Similar to the detergent treatments, two fabric softener treatments (cationic surfactant) were also included in the outdoor studies. Each treatment consisted of Downy fabric softener being applied to an area at the rate of 5.7 ml fabric softener per square meter. One application was made 1 day prior to inoculation (Fabric softener 1 day prior) and the other was applied 15 minutes after inoculation (Fabric softener post).

For all fabric softener and detergent treatments the product was diluted by a 1 to 50 ratio with water before application.

Additional treatments included: an inoculated infill system with no applied detergent, surfactant, or antimicrobial (Positive control) and an uninoculated infill system with no applied detergent, surfactant, or antimicrobial (Untreated control).

During the July and August studies another treatment was added (Broom post). Approximately 15 minutes after inoculation the treatment area was broomed in an effort to simulate the dragging of the surface with a stiff bristled broom.

The experimental design was a completely random design with three replications. The number of bacteria detected per cm2 was analyzed using analysis of variance and Fisher's Least Significant Difference (lsd) test at the 0.05 level. A lsd was not calculated when the F ratio was not significant at the 0.05 level.

EVALUATION

Experimental units were sampled at 1, 2, 3, and 4 hours after inoculation on Day 1 for all treatment areas. During the August study the positive control was sampled 15 min. after inoculation. Thereafter, all experimental units were sampled daily for a total of 3 days. The value used to represent the number of live bacteria at a given sampling time is the average of three sub-samples taken for each sampling time at different locations for each experimental unit. While this method sampled both the fibers and the surface crumb rubber, the amount of crumb rubber being tested by this method was small. So in addition to the petri plate method, at each sampling time a crumb rubber sample was also taken from selected treatments using a sterile test tube, 5 ml sterile distilled water was added to the crumb rubber in the test tube and shaken. A 0.1 ml subsample of the liquid was plated onto 85 mm BP agar plates and spread using a sterile inoculating loop. All plates were parafilmed and incubated at 36°C. After 48 hours, colonies on the plates were counted.

During the outdoor portions of this study, surface temperature and relative humidity were monitored using a WatchDog 100 Series data logger (Spectrum Technologies, Inc. Plainfield, IL).

Results and Discussion

Indoor Study

S. aureus survived for a number of days on almost all inoculated treatments including the Kentucky bluegrass sod (Tables 2 and 3). Treatments varied in their effectiveness at reducing *S. aureus* survival. The treatments that resulted in the quickest reduction of *S. aureus* survival were the surfactant and detergent applied 1 hr prior to inoculation and the detergent applied 15 minutes after inoculation. The antimicrobial alone and the antimicrobial plus surfactant did not result in as large a reduction in initial bacteria survival compared to the surfactant or detergent applied close to the time of inoculation. The antimicrobial did result in a significant reduction in survival rate after 24 hours compared to the untreated inoculated control; however, live bacteria was detected on all antimicrobial treatments for at least 216 hours after inoculation.

Table 2. Bacterial populations (bacteria per cm^2) detected on indoor synthetic surfaces and Kentucky bluegrass up to 288 hours (12 days) after bacterial application.

Treatment	Hours after bacterial application															
	1	2	3	4	24	48	72	96	120	144	170	194	216	240	264	288
	bacteria per cm^2															
Untreated control	0	-	-	0	0	0	0	0	0	0	0	0	0	0	-	-

Treatment	Hours after bacterial application															
	1	2	3	4	24	48	72	96	120	144	170	194	216	240	264	288
	bacteria per cm^2															
Low rate *S. aureus*	4.15	-	-	15.44	3.94	2.87	2.18	7.94	7.08	8.84	7.81	5.05	1.21	0.24	0.52	0.60
High rate *S. aureus*	45.92	-	-	45.92	17.21	16.58	14.39	9.35	14.76	4.30	5.17	9.32	4.30	0.97	1.25	2.10
Antimicrobial 1 hr prior[1]	12.76	6.17	4.29	8.88	0.92	0.24	0	0.07	0.12	0.01	0.07	0.07	0.01	0	-	-
Surfactant 1 hr prior[1]	1.53	0.51	0.05	0.04	0	0	0	0	0	0	0	0	0	0	-	-
Antimicrobial 25 days prior[1]	7.84	17.06	19.88	23.81	13.93	7.13	6.73	5.22	3.06	2.87	1.19	0.42	0.46	0.17	0.10	0
A+S 25 days prior[1]	12.33	10.07	11.48	7.19	1.16	0.83	0.22	0.34	0.07	0.04	0.04	0	0.02	0.02	-	-
Kentucky bluegrass	27.03	17.24	19.58	11.52	6.55	8.08	7.03	3.15	1.87	0.80	0.90	1.34	1.06	0.36	0.68	0.07
Detergent post[2]	1.65	0.41	0.41	0.05	0	0	0	-	-	-	-	-	-	-	-	-
Detergent 1.5 hr prior[2]	0.60	0.39	0.07	0.04	0	0	0	-	-	-	-	-	-	-	-	-
lsd (0.05)	7.6	7.7	5.2	5.5	4.1	4.4	6.7	5.5	9.0	3.3	2.0	NS[3]	NS	NS	NS	NS

[1]Treatment applied at a rate equal to 1.3 liters per m^2 (1640 gallons per American football field

[2]Treatment applied at a rate equal to 25 ml per m^2 (35 gallons product per American football field

[3]Not significant

Table 3. Percent viable bacteria detected on indoor synthetic surfaces and Kentucky bluegrass up to 288 hours (12 days) after bacterial application.

Treatment	Hours after bacterial application															
	1	2	3	4	24	48	72	96	120	144	170	194	216	240	264	288
	% of viable bacteria															
Untreated control	0	-	-	0	0	0	0	0	0	0	0	0	0	0	-	-
Low rate *S. aureus*	25.94	-	-	96.50	24.63	17.94	13.63	49.63	44.25	55.25	48.81	31.56	7.56	1.50	3.28	3.73
High rate *S. aureus*	29.44	-	-	29.44	11.03	10.63	9.22	5.99	9.46	2.76	3.31	5.97	2.76	0.62	0.80	1.35
Antimicrobial 1 hr prior[1]	8.18	3.96	2.75	5.69	0.59	0.15	0	0.04	0.08	0.01	0.04	0.04	0.01	0	-	-
Surfactant 1 hr prior[1]	0.98	0.33	0.03	0.03	0	0	0	0	0	0	0	0	0	0	-	-
Antimicrobial 25 days prior[1]	5.03	10.94	12.74	15.26	8.93	4.57	4.31	3.35	1.96	1.84	0.76	0.27	0.29	0.11	0.07	0
A+S 25 days prior[1]	7.90	6.46	7.36	4.61	0.74	0.53	0.14	0.22	0.04	0.03	0.03	0	0.01	0.01	-	-
Kentucky bluegrass	17.33	11.05	12.55	7.38	4.20	5.18	4.51	2.02	1.20	0.51	0.58	0.86	0.668	0.23	0.43	0.04
Detergent post[2]	1.06	0.26	0.26	0.03	0	0	0	-	-	-	-	-	-	-	-	-
Detergent 1.5 hr prior[2]	0.38	0.25	0.04	0.03	0	0	0	-	-	-	-	-	-	-	-	-

[1]Treatment applied at a rate equal to 1.3 liters per m^2 (1640 gallons per American football field)

[2]Treatment applied at a rate equal to 25 ml per m^2 (35 gallons product per American football field)

The survival rate for the bacteria detected on the crumb rubber alone was lower than detected using the petri plate method where predominantly fibers were being tested (Table 4). Although a small amount of living bacteria was found at 120 hours on the untreated positive control, with few exceptions most treatments had no detectable bacteria after 24 hours.

Table 4. Bacterial populations (number of live bacteria) detected on 0.2 g sample of ambient crumb rubber.

Treatment	Hours after bacterial appication									
	1	2	3	4	24	48	72	96	120	144
	bacteria per sample									
High rate *S. aureus*	23.18	-	-	50.96	0	0	0	0	0.04	0
Antimicrobial 1 hr prior[1]	0	0	0	0	0	0	0	0	0	0
Surfactant 1 hr prior[1]	6.06	0.10	1.29	8.47	0	0	0	0	0	0
Antimicrobial 25 days prior[1]	0	0	0	0	0	0	0	0	0	0
A+S 25 days prior[1]	18.09	0	0	0	0	0	0.09	0.12	0	0

Treatment	Hours after bacterial appication									
	1	2	3	4	24	48	72	96	120	144
	bacteria per sample									
Detergent post[2]	0.10	0	0.04	0	0	0	0	0	0	0
Detergent 1.5 hr prior[2]	0.04	0.14	0.07	0	0	0	0	0	0	0

[1]Treatment applied at a rate equal to 1.3 liters per m^2 (1640 gallons per American football field)

[2]Treatment applied at a rate equal to 25 ml per m^2 (35 gallons per American football field

Outdoor Studies

For all three of the studies conducted outdoors, levels of bacteria detected quickly dropped to very low levels (Tables 5 and 6). Typically, within 3 hours after applying bacteria to the surfaces, the number of bacteria detected was below 0.1 colonies per square centimeter for most treatments. On seven of the rating times, the Kentucky bluegrass treatment had bacteria counts higher than the positive control. This may be because the natural turf does not reach the very high surface temperatures that are achieved on the synthetic systems (McNitt, 2005).

Initially, two experimental times were chosen to represent varying environmental conditions. A mild period in May was selected when the temperatures were moderate and the skies were overcast (lower light). A second period in July was selected during a time of higher temperatures and clear skies. *S. aureus* survival was greatest during the May study. In fact, survival was so low in July that we decided to repeat the study during similar weather conditions in August.

S. aureus levels decreased more rapidly in the July and August experiments than in the May experiment. Surface temperature data collected during these experiments is reported in Table 8. Surface temperatures of the synthetic turf exceeded levels known to be lethal to *S. aureus* within two hours of inoculation during the July experiment (Baird-Parker, 1990). Surface temperatures did not reach levels considered to be outside of the growth range of *S. aureus* during the duration of the May experiment. However, surface temperatures were far above the optimal temperature for *S. aureus* during significant portions of all three outdoor experiments.

Table 5. Bacterial populations (bacteria per cm^2) detected on synthetic and natural playing surfaces after inoculation.

May 8

Treatment	Hours after bacterial application							
	1	2	3	24	48	72	96	144
	bacteria per cm^2							
Untreated control	0	0	0	0	0	0	0	0
Kentucky bluegrass	0.95	0.26	0.15	0.1	0.02	0	0.05	0
Fabric softener 1 day prior[1]	0.87	0.07	0	0.04	0.12	0.02	0.04	0
Detergent 1.5 hour prior[2]	1.22	0.04	0.02	0	0.02	0.07	0.04	0
Detergent 1.5 hour prior[1]	0.8	0.1	0	0.05	0.26	0.07	0.04	0
Detergent 1 day prior[2]	0.47	0.02	0	0	0.04	0.02	0.02	0
Antimicrobial + surfactant[3]	4.05	0.17	0.04	0.09	0	0.04	0.05	0
Detergent post[1]	0.24	0	0	0	0.04	0.05	0.02	0
Fabric softener post[1]	0.04	0	0.02	0	0.09	0.02	0.07	0.02
Positive control	1.31	0.04	0.02	0.12	0.07	0.02	0.02	0
lsd (0.05)	0.80	0.11	NS[4]	NS	NS	NS	NS	NS

July 8

Treatment	Hours after bacterial application					
	1	2	3	24	48	72
	bacteria per cm^2					
Untreated control	0	0	0	0	0	0
Kentucky bluegrass	0.36	0.05	0.68	0.02	0.07	0
Fabric softener 1 day prior[1]	0.04	0	0.15	0.05	0	0
Detergent 1.5 hour prior[2]	0.02	0.04	0.04	0	0	0
Detergent 1.5 hour prior[1]	0	0	0	0.07	0.02	0

Treatment	Hours after bacterial application					
	1	2	3	24	48	72
	bacteria per cm^2					
Detergent 1 day prior[2]	0.12	0.04	0.05	0.12	0	0
Antimicrobial + surfactant[3]	0.22	0.02	0.04	0.05	0	0
Broom post	0	0.09	0.02	0.04	0.09	0
Detergent post[1]	0.12	0.02	0.1	0.18	0.02	0
Fabric softener post[1]	0.05	0	0.07	0.08	0.02	0
Positive control	0.29	0.05	0.09	0.15	0.07	0
lsd (0.05)	NS	NS	NS	NS	NS	NS

August 8

Treatment	Hours after bacterial application						
	0.25	1	2	3	24	48	72
	bacteria per cm^2						
Untreated control	-	0	0	0	0	0	0
Kentucky bluegrass	-	0.34	0	0.15	0.05	0.22	0
Fabric softener 1 day prior[1]	-	0	0	0	0.09	0	0
Detergent 1.5 hour prior[2]	-	0.07	0.04	0	0.02	0	0
Detergent 1.5 hour prior[1]	-	0.02	0	0	0	0.02	0
Detergent 1 day prior[2]	-	0.09	0	0	0	0.07	0
Antimicrobial + surfactant[3]	-	0	0.02	0	0	0	0
Broom post	-	0.12	0.02	0	0	0	0
Detergent post[1]	-	0	0	0	0.02	0	0
Fabric softener post[1]	-	0.04	0	0	0.02	0	0
Positive control	1.07	0.02	0.12	0	0.04	0.02	0
lsd (0.05)	-	NS	0.05	0.07	NS	NS	NS

[1]Treatment applied at a rate equal to 5.7 ml per m^2 (8 gallons product per American football field)

[2]Treatment applied at a rate equal to 14.3 ml per m^2 (20 gallons product per American football field)

Treatment was applied 25 days (May and July) or 14 days (August)prior to application of bacteria at a rate equal to 1.3 liters per m^2 (1640 gallons per American football field)

[4]Not significant

Table 6. Percent of viable bacteria detected on synthetic and natural playing surfaces after inoculation

May 8

Treatment	Hours after bacterial application							
	1	2	3	24	48	72	96	144
	% of viable bacteria							
Untreated control	0	0	0	0	0	0	0	0
Kentucky bluegrass	5.94	1.63	0.94	0.63	0.13	0	0.31	0
Fabric softener 1 day prior[1]	5.44	0.44	0	0.25	0.75	0.13	0.25	0
Detergent 1.5 hour prior[2]	7.63	0.25	0.13	0	0.13	0.44	0.25	0
Detergent 1.5 hour prior[1]	5.00	0.63	0	0.31	1.63	0.44	0.25	0
Detergent 1 day prior[2]	2.94	0.13	0	0	0.25	0.13	0.13	0
Antimicrobial + surfactant[3]	25.31	1.06	0.25	0.56	0	0.25	0.25	0
Detergent post[1]	1.50	0	0	0	0.25	0.31	0.31	0
Fabric softener post[1]	0.25	0	0.13	0	0.56	0.13	0.13	0.13

Treatment	Hours after bacterial application							
	1	2	3	24	48	72	96	144
	% of viable bacteria							
Positive control	8.19	0.25	0.13	0.75	0.44	0.13	0.13	0

July 8

Treatment	Hourse after bacterial application					
	1	2	3	24	48	72
	% of viable bacteria					
Untreated control	0	0	0	0	0	0
Kentucky bluegrass	2.25	0.31	4.25	0.13	0.44	0
Fabric softener 1 day prior[1]	0.25	0	0.94	0.31	0	0
Detergent 1.5 hour prior[2]	0.13	0.25	0.25	0	0	0
Detergent 1.5 hour prior[1]	0	0	0	0.44	0.13	0
Detergent 1 day prior[2]	0.75	0.25	0.31	0.75	0	0
Antimicrobial + surfactant[3]	1.38	0.13	0.25	0.31	0	0
Broom post	0	0.56	0.13	0.25	0.56	0
Detergent post[1]	0.75	0.13	0.63	1.13	0.13	0
Fabric softener post[1]	0.31	0	0.44	0.50	0.13	0
Positive control	1.81	0.31	0.56	0.94	0.44	0

August 8

Treatment	Hourse after bacterial application						
	0.25	1	2	3	24	48	72
	% of variable bacteria						
Untreated control	-	0	0	0	0	0	0
Kentucky bluegrass	-	2.13	0	0.94	0.31	1.38	0
Fabric softener 1 day prior[1]	-	0	0	0	0.56	0	0
Detergent 1.5 hour prior[2]	-	0.44	0.25	0	0.13	0	0
Detergent 1.5 hour prior[1]	-	0.13	0	0	0	0.13	0
Detergent 1 day prior[2]	-	0.56	0	0	0	0.44	0
Antimicrobial + surfactant[3]	-	0	0.13	0	0	0	0
Broom post	-	0.75	0.13	0	0	0	0
Detergent post[1]	-	0	0	0	0.13	0	0
Fabric softener post[1]	-	0.25	0	0	0.13	0	0
Positive control	6.69	0.13	0.75	0	0.25	0.13	0

[1]Treatment applied at a rate equal to 5.7 ml per m^2 (8 gallons product per American football field)

[2]Treatment applied at a rate equal to 14.3 ml per m^2 (20 gallons product per American football field)

[3]Treatment was appplied 25 days (May and July) or 14 day (August) prior to application of bacteria at a rate equal to 1.3 liters per m^2 (1640 gallons per American football field)

During the August 2008 study, at one hour after inoculation, low levels of bacteria were detected in all but three of the inoculated treatments, with the Kentucky bluegrass and brushing treatments having the highest numbers of bacteria (Tables 5 and 6). Neither of these treatments received any type of antimicrobial or disinfecting products. Within three hours of inoculation, bacterial populations had dropped considerably with most treatments measuring zero survival. Trace amounts of bacteria were detected in several treatments at 24 and 48 hours after inoculation. By 72 hours after the application of bacteria, no *S. aureus* was detected in any treatment.

The survival of *S. aureus* on synthetic turf and Kentucky bluegrass surfaces is likely affected by surface temperature. However, ultraviolet (UV) radiation may be as much of a limiting factor in the survival of *S. aureus* as the temperatures reported in this study

(Baird-Parker, 1990; Hardjawinata et al, 2005). While surface temperatures on 26 Aug exceeded the upper limit for the survival of *S. aureus* (Table 8), most of the bacteria appeared to be non-viable prior to these high temperatures. This may be due in large part to outdoor UV radiation. Bright sunshine was present during the daylight hours during the first 48 hours of the August study. *S. aureus* has been shown to be inhibited or destroyed by relatively short exposure to UV light (Hardjawinta et al, 2005; Silva et al, 2003). It should be noted that there was also a light rain on the final day that data was collected during the August study. Future research should consider the effects of precipitation or irrigation on *S. aureus*.

Data collected during the indoor study indicated that the survival rate for bacteria was generally lower on the crumb rubber alone compared to the petri plate method which sampled fibers to a greater degree. It was decided that sampling of the crumb rubber alone during the outdoor study would not yield useful results after one or two days. In retrospect, this data may well have proved useful especially during the July testing period (Table 7). Future studies should continue to sample for a longer period of time.

The number of bacteria detected when sampling the crumb rubber alone was generally small in comparison to the amount detected during the indoor study and viability does seem to be less on the crumb rubber alone compared to the petri plate method that tests both fibers and surface crumb rubber. Certainly during the May and August testing period the amount of bacteria detected on the crumb rubber is small or zero. It would have been interesting to see the bacteria count for 48 and 72 hours for the July rating date but those data are not available.

Table 7. Bacterial populations (bacteria per cm²) detected on ambient crumb rubber samples after inoculation

May 8

Treatment	Hours after bacterial application				
	1	2	3	24	48
	bacteria per sample				
Negative control	0	0	0	0	0
Antimicrobial + Surfactant[1]	0	0.02	0	0.14	0.02
Positive control	0.15	0.03	0	0.02	0.02

July 8

Treatment	Hours after bacterial application			
	1	2	3	24
	bacteria per sample			
Negative control	0	0	0	0
Antimicrobial + Surfactant[1]	0.59	1.04	0.10	0.53
Positive control	0.07	0.51	0.73	0.48

August 8

Treatment	Hours after bacterial application			
	1	2	3	24
	bacteria per sample			
Negative control	0	0	0	0
Antimicrobial + Surfactant[1]	0	0	0	0
Positive control	0.02	0	0	0

[1]Treatment applied 25 days (May and July) or 14 days (August) prior to application of bacteria at a rate equal to 1.3 liters per m^2 (1824 gallons per American football field)

Table 8. Maximum surface and air temperatures during the duration of outdoor S. aureus survival experiments

Date	Maximum temperature (°C)	
	Surface[1]	Air[2]
27 May	40.6	26.7
28 May	37.6	18.4

Date	Maximum temperature (°C)	
	Surface[1]	Air[2]
29 May	43.1	22.8
30 May	42.6	27.2
31 May	43.1	26.7
1 Jun	39.6	23.9
2Jun	31.1	24.5
30 Jul	54.1	29.5
31 Jul	50.6	29.5
1 Aug	51.6	29.5
2 Aug	51.1	28.9
3 Aug	49.6	25.6
4 Aug	51.2	27.8
25 Aug	NA	25.6
26 Aug	50.1	26.7
27 Aug	47.1	25.6
28 Aug	19.6	21.2

[1]Temperature determined by datalogger placed directly on synthetic turf surface.
[2]Temperature obtained from Pennsylvania State Climatologist website, Penn State Department of Meteorology

Conclusions

Under non-extreme temperature and very limited light conditions present during the indoor portion of this study, *S. aureus* survived on both synthetic and natural turfgrass for multiple days. However, the bacteria do not appear to thrive under these conditions as the numbers of surviving bacteria decrease significantly with time. *S. aureus* survival seems to be greatest on the fibers compared to the crumb rubber infill. Commercially available antimicrobial treatments as well as detergent significantly decreased the survival rate of *S. aureus* present on these surfaces indoors although every experimental unit inoculated tested positive for the presence of *S. aureus* for the first 4 hours and a number were still positive 9 days after inoculation. Commercially available detergent and the cationic surfactant SportsClean applied around the time of inoculation resulted in no live bacteria detected after 24 hours.

When *S. aureus* is applied to outdoor surfaces under conditions of higher temperatures in the presence of UV light, the bacterial survival rate was much lower. It is difficult to draw conclusions regarding the effectiveness of various treatments in an outdoor environment because the bacteria do not appear to survive very long under these conditions whether treatments were applied or not, but both detergent and fabric softener applied to the surface around the time of bacterial inoculation seem to reduce *S. aureus* survival somewhat. However, exposure to UV light and higher temperature seem to be the most effective disinfectant under the conditions of this experiment. It should be noted that *S. aureus* survival rate on a common turfgrass species used for athletic fields in the Northern United States was comparable to the survival rate on synthetic turf when no disinfectants were applied.

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