

A Survey of Microbial Populations in Infilled Synthetic Turf Fields

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Introduction

Staphylococcus aureus is a bacterium that is a common inhabitant of human skin and can cause various types of skin or soft tissue infections (Marples, et al, 1990). *S. aureus* has also been implicated in certain types of food poisoning (Bennet and Lancette, 1998) and in serious medical problems such as toxic shock syndrome. Strains of *S. aureus* that are resistant to common antibiotics are becoming more common, particularly in medical settings. There have been reports recently of methicillin-resistant *S. aureus* causing infection in athletes (Begier, et al, 2004). With the increase in athlete infections, there is growing concern regarding the role of infilled turf systems (Seppa, 2005). While there is some indication that the source of these bacteria may be more closely associated with locker room activity than with the infill system (Begier, et al, 2004; Kazakova, et al, 2005), conclusive evidence is not currently available.

The objective of this survey was to determine the microbial population of several infilled synthetic turf systems as well as natural turfgrass fields. In addition, other surfaces from public areas and from an athletic training facility were also sampled. Colonies suspected to be *S. aureus* were positively or negatively identified.

Materials & Methods

Sample Collection

All samples in this study were collected between June 15 and June 30, 2006. Infilled synthetic turf systems were located at facilities in Pennsylvania and were in use by all levels of play ranging from elementary to professional athletes. Crumb rubber samples were collected from both a 'high use' and a 'low use' area of each field. A 'high use' area typically was a goal mouth or, for a football only field, an area between the 30- and 40-yard lines between the hash marks. A 'low use' area was typically an area toward the edge of the field (but within the field of play) or an end zone. Approximately 2-3 ml of crumb rubber were removed from each area of the field using a sterile test tube inserted directly into the infill. Pile fiber samples were also collected from many fields by clipping several fibers from the backing and transferring the fibers to a sterile test tube. Samples were stored in a cooler and processed as soon after collection as possible.

Sample Processing

Approximately 0.075 g of crumb rubber was transferred to a test tube containing 10 ml sterile 0.1% peptone broth. The sample was agitated for 30 seconds. Serial dilutions of each sample were plated up to 10^{-3} on both R2A agar for total organism populations and Baird-Parker agar, a selective media for *Staphylococcus* (Bennet and Lancette, 1998). Duplicate platings were made for each media and each dilution. Petri plates were parafilmed and incubated at room temperature and colony counts were made 72 hours after processing. Samples on Baird-Parker agar were also observed again after 5 days. Calculations were then made to determine the number of colony forming units (CFU) per gram of crumb rubber.

For comparison purposes, soil samples were also collected from a native soil and a sand based natural turfgrass athletic field. Samples were processed in the same manner as the crumb rubber samples with 0.2 grams of soil being used for processing.

Sampling of Other Surfaces

Samples were collected from common surfaces in public areas as well as from various surfaces in an athletic training area. Samples were collected by swabbing surfaces with sterile cotton swabs. Random individuals were also tested by swabbing hands and/ or face. Both R2A and Baird-Parker agar plates were wiped with the sterile swabs. Plates were incubated at room temperature and colony counts were conducted after 72 hours for R2A agar and again at 5 days for Baird-Parker media.

Identification of *Staphylococcus aureus* colonies

Gram stains and latex agglutination tests (Essers and Radebold, 1980) were performed on colonies suspected of being *S. aureus*. Several potential *S. aureus* colonies isolated from hand and facial swabs were also included in the testing.

Results

Field Samples

The results of total microbial populations are shown in Table 1. While microbes exist in the infill media the number was low compared to natural turfgrass field soils. It should be remembered that microbes tend to be present on most surfaces humans come in contact with and the simple presence of microbes should not be cause for concern. In fact, many products on the market claim to boost the microbial populations of natural turfgrass soils with higher microbial populations considered to be beneficial.

Table 1. Colony forming units (CFU) detected on R2A media per gram of crumb rubber.

Field ID# ¹	Product	CFU/ g crumb rubber
1H	100% crumb rubber	20200
1L	100% crumb rubber	32667
2H	Crumb rubber - Sand mix	3533
2L	Crumb rubber - Sand mix	133
3H	Crumb rubber - Sand mix	3467
3L	Crumb rubber - Sand mix	13133
4H	100% crumb rubber	9467
4L	100% crumb rubber	30267
5H	Crumb rubber - Sand mix	18867
5L	Crumb rubber - Sand mix	8333
6H	Crumb rubber - Sand mix	267
6L	Crumb rubber - Sand mix	28333
7H	Crumb rubber - Sand mix	4800
7L	Crumb rubber - Sand mix	55333
8H	Crumb rubber - Sand mix	4867
8L	Crumb rubber - Sand mix	24133
9H	100% crumb rubber	9800
9L	100% crumb rubber	17867
10H*	Crumb rubber - Sand mix	0
10L*	Crumb rubber - Sand mix	67
11H	Crumb rubber - Sand mix	33200
11L	Crumb rubber - Sand mix	28000
12H	100% crumb rubber	333
12L	100% crumb rubber	800
13H*	100% crumb rubber	267
13L*	100% crumb rubber	67
14H	100% crumb rubber	8267
15H	Crumb rubber - Sand mix	8600
15L	Crumb rubber - Sand mix	3867
16H*	100% crumb rubber	200
16L*	100% crumb rubber	0
17H	Crumb rubber - Sand mix	0
17L	Crumb rubber - Sand mix	7267
18H	100% crumb rubber	5000
18L	100% crumb rubber	5533
18H	Crumb rubber - Sand mix	53067
18L	Crumb rubber - Sand mix	80000
20H	Crumb rubber - Sand mix	54333
20L	Crumb rubber - Sand mix	8867
Native soil (silt loam)		259500
Sand based soil		309500

¹ H = sample collected from higher use area of field

L = sample collected from lower use area of field

* = sample collected from indoor field

Pile fiber samples were also collected from several fields. CFUs for fiber samples range from 200-2933 CFUs per fiber sample (2 fibers approximately 1 cm long) indicating that the fibers alone generally exhibited lower microbial populations compared to the infill.

Microbial colonies isolated from field samples generally included both fungi and bacteria. Some fields had predominantly one organism type while other fields contained a variety of organisms. In order to positively identify the presence of *S. aureus*, three procedures were used. No colonies isolated from any crumb rubber or fiber samples tested positive for *S. aureus* via selective media, gram stain or latex agglutination tests.

Other Surfaces

Surfaces other than athletic playing surfaces were tested for the presence of microbes and *S. aureus*. These surfaces are not granulated and thus the results are listed in Table 2 as total colony number as opposed to CFU per gram of granulated material.

Table 2. Number of colonies per swab detected on R2A media from various sources in public spaces and an athletic training facility.

<u>Source</u>	<u>Colony number</u>
<u>Public areas</u>	
Computer mouse	>600
Elevator button	155
Outside door handle	80
Computer keyboard	33
<u>Athletic training facility</u>	
Cold pool	24
Blocking pads*	130
Sauna	536
Football*	142
Weight equipment 1*	62
Weight equipment 2*	414
Towel hamper	103
Stretching table	14
Used towels*	29
<u>Trash can for drink cups</u>	<u>205</u>

*Sampled immediately after use

Table 3. Surfaces that tested positive (+) for *S. aureus* colonies per swab detected.

<u>Source</u>	<u>Colony number</u>
<u>Public areas</u>	
Human hands	+
Human faces	+
Computer mouse	-
Elevator button	-
Outside door handle	-
Computer keyboard	-
<u>Athletic training facility</u>	
Natural turfgrass playing field	-
Synthetic turf playing field	-
Cold pool	-
Blocking pads*	+
Sauna	-
Football*	-
Weight equipment 1*	+
Weight equipment 2*	+
Towel hamper	-
Stretching table	+
Used towels*	+
<u>Trash can for drink cups</u>	-

*Sampled immediately after use

Microbial colonies isolated from surfaces included a mixture of fungi and bacteria. Colonies from the trash can were predominantly fungi. While not specifically identified, all colonies from the sauna swab appeared to be the same. *S. aureus* was positively identified from several samples including towels, blocking pads, weight equipment, and the stretching table. In addition, *S. aureus* was positively identified from every facial and hand swabs done.

Discussion

Considering the data in Table 1, the number of total colonies detected varied considerably between sites. In most cases, the number of total microbes at a given site was similar for both high use and low use areas of the field. In general, indoor fields tended to have lower overall microbial populations (0-7267 CFU) than outdoor fields. At one facility where indoor and outdoor fields were sampled on the same day, the indoor fields contained 0-67 CFU/g crumb rubber, while the outdoor field contained 2.8-3.3x10⁴ CFU/g crumb rubber. While indoor fields represent only 20% of the fields sampled in this study a consistent trend is apparent.

Total microbial populations for the two natural turf athletic fields were an order of magnitude higher than populations for the infill systems with the highest numbers. Also, observationally, there appeared to be a greater diversity in the types of organisms isolated from soil samples than crumb rubber samples. However, no specific determinations of any cultures were made other than to positively or negatively identify *S. aureus*.

One factor that may influence total microbial populations of infill surfaces is use. Of the 11 fields with at least one subsample having greater than 1x10⁴ CFU/g crumb rubber, one of those fields had been heavily used within 7 days of sampling and two fields had been used within 24 hours of sampling. Tracking microbial population fluctuations of a field over time may be of interest.

It is perhaps not surprising that no *S. aureus* colonies were found on any fields, given that the temperature range for growth of *S. aureus* is 7-48°C, with the optimal temperature for growth being 37°C (Baird-Parker, 1990). Surface temperatures of infill surfaces outdoors often exceed the temperature range of *S. aureus* (McNitt, 2005). However, high surface temperatures do not explain the relatively low numbers of total microbes on indoor playing surfaces. These low numbers may, in part, be explained by the permanently very low moisture content of indoor infilled surfaces.

In conclusion, there are generally lower numbers of total microbes present in the infill or fibers of the synthetic turf systems tested compared to natural turfgrass rootzones and *Staphylococcus aureus* bacterium were not found on any of the playing surfaces. *Staphylococcus aureus* bacterium were found on towels and other devices used to train athletes.

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