[Primary Care]

Microbiota Found in Protective Athletic Mouthguards

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Background: Protective athletic mouthguards (PAM) produce oral mucosal injuries and may be associated with other systemic conditions.

Hypothesis: With wear, PAM become contaminated by a range of microorganisms. The number of microorganisms in PAM can be reduced by daily use of an antimicrobial solution.

Study Type: Case series.

Study Design and Methods: Sixty-two division I football player volunteers were divided into 4 groups, using PAM for varying lengths of time before surrendering them for microbial analysis. One group had their PAM soaked in an antimicrobial solution between uses. The PAM were analyzed qualitatively and quantitatively for bacteria, yeasts, and fungi, using previously accepted methods.

Results: The 62 football players surrendered a total of 81 PAM for microbial analysis. The PAM yielded 154 gram-positive cocci, 150 gram-positive bacilli, 21 gram-negative cocci, 31 gram-negative bacilli, 22 yeasts, and 107 molds. The most common species of gram-positive cocci were *Staphylococcus* spp. and *Micrococcus* spp. Only 3 PAM (4%) were positive for *Staphylococcus aureus*. The most common species of gram-positive bacilli were *Brevibacterium* spp. and *Cellulomonas* spp. The most common species of yeasts were *Candida parapsilosis* and *Rhodotorula mucilaginosa*, while the most common species of mold were *Cochliobolus* spp. and *Penicillium chrysogenum*. Soaking the PAM in an antimicrobial solution between uses substantially reduced the numbers of microorganisms.

Conclusions: Substantial microbial contamination of PAM occurs with use. The microbial load can be reduced by soaking in an antimicrobial solution between uses.

Clinical Relevance: PAM are contaminated by microorganisms that have the potential to produce oral and systemic diseases. They should be sanitized daily and changed when they become sharp and/or jagged.

Keywords: protective athletic mouthguards; contamination; disease transmission; oral infections; systemic infections; microorganisms; mouthguard care

Protective athletic mouthguards (PAM) or mouthpieces provide protection for the teeth during contact sports.[4,12-14,16,18,19] These results have prompted the American Dental Association to support the use of PAM for all contact sports. Football teams at all levels have been among the most compliant with the association's recommendations. However, the best practices for sanitization and changing these devices are unknown.[2] Many teams allow players to store and replace their PAM as they deem necessary.[1,3,5,15] Recent studies of hockey players,[8] football players, and medical students[6] have shown that PAM harbor a range of pathogenic and opportunistic bacteria, yeasts, and molds.[17] Ultrastructural analyses by scanning electron microscopy reveal that the microorganisms can be found both on the surfaces and in the porosities of the polymerized PAM material (Figures 1 and 2).

In previous studies of PAM, the 2 most frequently isolated gram-positive cocci (*Staphylococcus aureus* and *Staphylococcus epidermidis*) were not only prominent skin pathogens but also associated with endocarditis, pericarditis, pneumonia, osteomyelitis, food intoxication, and athletic equipment contamination.[6,8] Other species of bacteria and fungi isolated from the football and hockey PAM were associated with myriad diseases, such as meningitis, urinary tract infections, peritonitis, periodontal disease, wound infections, and exercise-induced asthma.[9,10]

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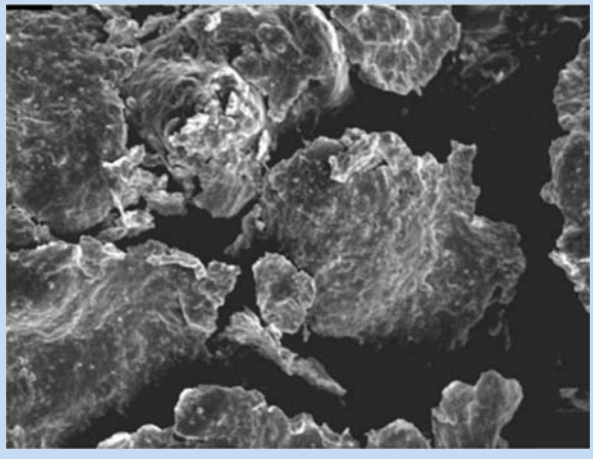

Figure 1. Scanning electron microscopy of a typical protective athletic mouthguard. Note the large crevices that allow for deep penetration of microorganisms and produce difficulties in the mechanical removal of the microbes. Original magnification, 1000×.

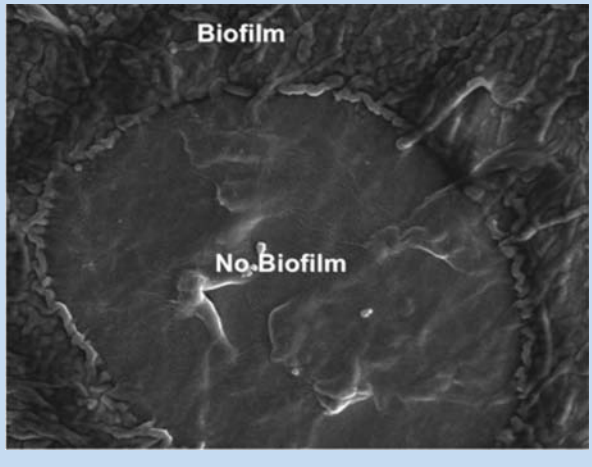

Figure 2. Scanning electron microscopy of a protective athletic mouthguard demonstrating large accumulations of a variety of microorganisms forming a complex network (biofilm), as well as areas that appear completely free of any microbes (no biofilm). Original magnification, 1000×.

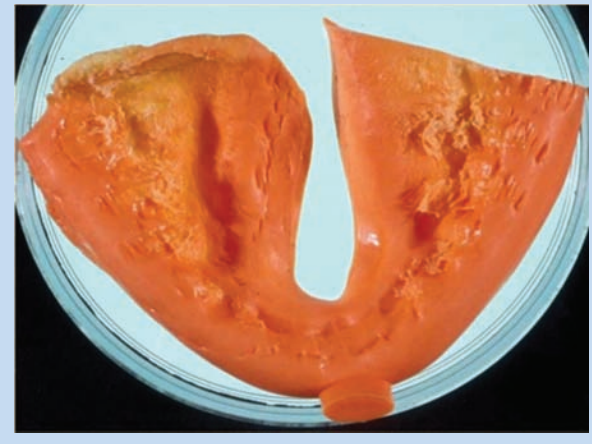

Figure 3. Typical protective athletic mouthguard after a season of wear. Note the rough, jagged edges capable of creating a microbial portal of entry into the tissues.

Figure 4. Typical position of the protective athletic mouthguard in between plays (arrow), allowing for substantial environmental contamination.

While both football and hockey PAM studies do not represent the total microbial population found on PAM, they are sufficient to warrant genuine concern. Also, the condition of PAM after wear may facilitate disease transmission.[6,8-10] The jagged, sharp areas of the posterior regions of typical PAM are in close proximity to the pterygoid plexus of veins and, therefore, the entire circulatory system (Figure 3). A recent study over the course of a season confirmed that football mouthguards increased the number and severity of oral mucosal injuries.[9] Previous studies found that some commercially available PAM were contaminated before use.[6,8]

PAM storage is also an important factor to consider. Rules dictate that the football and hockey PAM be in the players' mouths during play; a typical location of the PAM between plays is often extraoral (Figure 4). These tendencies facilitate the contamination of the device with a range of environmental microbes.

PAM are porous and can become contaminated, emphasizing the need to evaluate the *in vivo* spectra of microorganisms that may be found within such devices. The following study was performed to address these questions: (1) Do PAM become contaminated by a range of microorganisms with wear? (2) Can the microorganisms found on and in PAM be either eliminated or significantly reduced (10^3 reduction) using a sanitizing formulation (NitrAdine, MSI Laboratory, Vaduz, Liechtenstein)?

METHODS

Participants

Sixty-two athletes from a division I football team were voluntarily recruited at the beginning of the fall season. After informed consent was obtained, the athletes were provided the PAM of their choice (boil-and-bite or custom-made) and randomized into 4 groups[11]:

- *Group A:* Players were not expected to compete in games but did practice. This group wore the same PAM throughout the entire season.
- *Group B:* Players were expected to practice and compete in games. This group also wore the same PAM for the entire season.
- *Group C:* Players practiced and competed in games but wore the same PAM until midseason only. At that time, the PAM were retrieved and immediately transported to a laboratory for microorganism analysis (Oklahoma State University Center for Health Sciences Forensic Sciences and Infectious Disease Laboratory, Tulsa, Oklahoma). Each athlete then received a new mouthguard of his choice for the remainder of the season.
- *Group D:* Players practiced and competed in games and wore the same PAM until midseason. However, their PAM were placed into a sanitizing solution (NitrAdine) after each practice or game until they were worn again. At midseason, the PAM were sent to the same laboratory for microorganism analysis. Each athlete received a new mouthguard for the remainder of the season, which was sanitized as previously described.

Note that if the mouthguard was lost or was damaged beyond use at any time during this study, it was replaced immediately. In the course of this study, if an individual submitted multiple mouthguards for analysis, the mouthguard of record was the one worn for the longest period. Eleven medical student volunteers, who wore similar PAM for a minimum of 12 hours, acted as controls for this study.[6,8]

Microbial Methods

The PAM were cultured for microorganisms using the same techniques previously described for hockey and football players' PAM.[6,8,9] The surfaces and depths of the pieces were sampled for fast-growing microorganisms.[6,8,9] All cultures were scored on the 0-4 intensity scale.[6,8,9]

Pure cultures of each isolate were identified using standard laboratory procedures, including a combination of selective and/or differential media, standard biochemical tests, and Analytical Profile Index procedures (BioMérieux Viteck Inc, Hazelwood, Missouri).

Mold isolates were identified by comparative DNA sequence analysis (Megablast; http://blast.ncbi.nlm.nih.gov/Blast.cgi) of the internal transcribed spacer region with published nonredundant database entries at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The internal transcribed spacer is located between the small subunit and large subunit ribosomal RNA genes in fungi.[20] Microbial growth/intensities were compared at midseason for football groups C (untreated) and D (treated). At the end of the season, all groups were analyzed to determine if treating the PAM with a sanitizing solution (NitrAdine) reduced the microbial load.

RESULTS

The 81 PAM yielded a total of 356 bacterial isolates, 22 yeast isolates, and 107 mold isolates for a total of 485 microbial isolates (Table 1). Changing the PAM midseason (Group C) did not substantially change the numbers of microorganisms. Group D were not significantly different from Group C. Regular treatment of PAM (Group D after midseason) with a sanitizing solution did produce a significant drop in the numbers of microbial isolates of all types ($P < 0.05$).

The most common species of gram-positive cocci were *Staphylococcus* spp. and *Micrococcus* spp., with only 3 isolates being *S aureus*. The most common species of gram-positive bacilli were *Brevibacterium* and *Cellulomonas*. The most common species of yeasts were *Candida parapsilosis* and *Rhodotorula mucilaginosa*, while the most common species of mold were *Bipolaris/Cochliobolus* spp. and *Penicillium chrysogenum* (Tables 2 and 3).

Changing the PAM at midseason reduced the bacterial loads in both groups C and D but not significantly (Table 1). Even more compelling is the reduction of bacterial isolates associated with daily sanitization (group D after midseason).

DISCUSSION

The results of this study support the contention that PAM become highly contaminated with use.[9,10] There were no significant differences between untreated regular (varsity) and reserve (redshirt and nonplaying freshmen) football players (Table 1). The untreated PAM from groups A, B, and C showed similar levels of contamination. Group D PAM was sampled at midseason and at season end. The difference between midseason collection and season-end collection was the timing of the last sanitizing treatment: The midseason PAM were last treated 48 hours before sampling, while the season end PAM were sampled immediately after treatment. This finding

Table 1. Football players' protective athletic mouthguards: Microbial data, n.

Source of PAM	PAM	Gram⁺ Cocci	Gram⁺ Bacillii	Gram⁻ Cocci	Gram⁻ Bacilli	Yeasts	Molds	Total	Isolates / PAM
Group A: Season end[a]	12	23	17	4	5	1	20	70	5.8
Group B: Season end[b]	10	15	28	1	5	3	16	68	6.8
Group C[c]									
Midseason	15	42	35	4	11	9	28	129	8.6
Season end	14	29	44	0	2	2	19	96	6.9
Group D[d]									
Midseason	16	42	14	12	5	6	20	99	6.2
Season end	14	3	12	0	3	1	4	23	1.6
Total	81	154	150	21	31	22	107	485	—
Isolates/PAM		1.90	1.85	0.26	0.38	0.27	1.32		5.98

[a]Reserve players (redshirts and freshmen): protective athletic mouthguards (PAM) worn all season—untreated.
[b]Regular players: PAM worn all season—untreated.
[c]Regular players: PAM changed midseason—untreated; PAM collected at season end—untreated.
[d]Regular players: PAM changed midseason—treated with NitrAdine after each use (however, these PAM were last treated 2 days before collection and after being worn in a game); PAM collected at season end—treated with NitrAdine after each use.

Table 2. Yeasts isolated from protective athletic mouthguards.

Yeast Species	Incidence
Candida albicans	2
C.famata	1
C.guilliermondii	1
C.parapsilosis	9
Rhodotorula mucilaginosa	8
Trichosporon asahii	1
Total	22

Table 3. Molds isolated from protective athletic mouthguards.

Mold Species	Incidence
Alternaria spp.	2
Arthrinium phaeospermum	1
Aspergillus peyronelii	2
Aspergillus unguis	1
Bipolaris spp. / *Cochliobolus* spp.	25
Cladosporium cladosporioides	1
Exserohilum rostratum	1
Fusarium spp.	2
Hypocreales spp.	2
Leptosphaerulina spp.	1
Penicillium chrysogenum	36
Phoma medicaginis	4
Rhizopus spp.	16
Undetermined mold	13
Total	107

suggests that rapid recontamination occurs and that there is a need for daily sanitizing.

Previous studies of this subject pool evaluated the clinical status of the players' oral cavities and found an increase in both the oral lesions and the severity as the season progressed.[9] The large number of bacteria and yeasts found in the present study support a relationship between the microbial-contaminated PAM and the oral lesions. Daily

sanitization appears to be beneficial, as consistent with studies of dentures showing marked reductions in the microbial loads.[7]

In conclusion, it appears that PAM become contaminated with wear but that contamination can be reduced with daily sanitization.

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