Significant changes in the skin microbiome mediated by the sport of roller derby

Diverse bacterial communities live on and in human skin. These complex communities vary by skin location on the body, over time, between individuals, and between geographic regions. Culture-based studies have shown that human to human and human to surface contact mediates the dispersal of pathogens, yet little is currently known about the drivers of bacterial community assembly patterns on human skin. We hypothesized that participation in a sport involving skin to skin contact would result in detectable shifts in skin bacterial communities. We conducted a study during a flat track roller derby tournament, and found that teammates shared distinct skin microbial communities before and after playing against another team, but that opposing teams' bacterial communities converged during the course of a roller derby bout. Our results are consistent with the hypothesis that the human skin microbiome shifts during activities involving human to human contact, and that contact sports provide an ideal setting in which to evaluate dispersal of microorganisms between people.

PeerJ PrePrints

Significant changes in the skin microbiome mediated by the sport of roller derby

James F. Meadow[1,*], Ashley C. Bateman[1], Keith M. Herkert[1,2] Timothy K. O'Connor[1,3] Jessica L. Green[1,4]

[1] Biology and the Built Environment Center, Institute of Ecology and Evolution, University of Oregon, Eugene, OR, USA

[2] Oregon Health & Science University, Portland, OR, USA

[3] Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, USA

[4] Santa Fe Institute, Santa Fe, NM, USA

[*] **Corresponding Author:**

James F. Meadow

5289 University of Oregon

Eugene, OR 97403-5289

Phone: (406) 370-7157

E-mail: jfmeadow@gmail.com

PeerJ PrePrints

Introduction

Microbial communities living on and in the human skin are diverse and complex. These communities, which vary greatly both within and among people, play an important role in human health and well-being. Skin microbial communities have been shown to mediate skin disorders, provide protection from pathogens, and regulate our immune system (Costello *et al.* 2009; Grice & Segre 2011; Human Microbiome Project Consortium 2012). Despite the importance of our skin microbiota, we still know very little about what shapes the distribution and diversity of the skin microbiome.

As for any other ecosystem, the composition of the skin microbiome is determined by some combination of two simultaneous ecological processes: the selection of certain microbial types by the skin environment and the dispersal of microbes from a pool of available species. Skin moisture, temperature, pH and exposure to ultraviolet light are all well documented environmental factors that affect skin microbial communities (Grice & Segre 2011). The microbial species available for dispersal onto the skin of any given individual likely stem from many sources including inanimate surfaces, people, pets, cosmetics, air and water (Capone *et al.* 2011; Costello *et al.* 2009; Dominguez-Bello *et al.* 2010; Fierer *et al.* 2008; Fujimura *et al.* 2010; Grice & Segre 2011; Hospodsky *et al.* 2012; Human Microbiome Project Consortium 2012; Kembel *et al.* 2012). Our current understanding of the relative contributions from these potential sources is nascent. Human to surface and human to human contact have long been acknowledged as strong vectors for microbial dispersal in the medical literature, which has been largely focused on culture-based detection of single-species pathogen transmissions (Boyce *et al.* 1997; Casewell & Phillips 1977; Hamburger Jr. 1947; Noble *et al.* 1976; Pessoa-Silva *et al.* 2004; Pittet *et al.* 2006). In these culture-based studies, handshaking, as well as hand-contact with other parts of the body and room surfaces, have been identified as strong vectors of health care service infections, such as with methicillin-resistant *Staphylococcus aureus* (MRSA) and *Klebsiella* spp. (Casewell & Phillips 1977; Davis *et al.* 2012; Pittet *et al.* 2006). Given that human contact with surfaces, and especially the skin surfaces of others, has been shown to transfer individual microbial taxa, activities which involve human to human contact could be assumed to result in the sharing of skin microbial communities.

Here we explore how activities involving human to human contact influence the skin microbiome. We use a contact sport, flat track roller derby, as a model study system. Flat track roller derby is an organized team sport, played worldwide, that involves individuals roller-skating in close proximity

PeerJ PrePrints

and making frequent contact with other players. Roller derby teams frequently engage in tournaments, where teams from different geographical locations come together to play, or 'bout' against one another for several days at a time. Flat track roller derby tournaments present an ideal setting in which to study the transmission of skin microbial communities during a full-contact sport for two main reasons. First, they provide an opportunity to assess if the skin microbiome from athletes that frequently come into contact with one another - members of the same team- have similar microbiomes. Second, they provide an opportunity to assess if skin microbiomes of athletes on opposing teams become more similar after competing against one another. Specifically, we addressed the following questions in our study: 1) Were players' skin microbiomes predicted by team membership; 2) Were team-specific skin microbiomes altered during a bout; and 3) Did opposing teams' skin microbiomes become more similar, or converge, after competing in a bout?

Materials and Methods

Flat-Track Roller Derby

For a full explanation of approved Womens Flat Track Derby Association rules, refer to www.wftda.com. Briefly, a bout consists of two 30-minute periods, where two competing teams, each composed of up to 4 "blockers" and 1 "jammer," circle a track with the goal of facilitating their own jammer in accumulating points. Points are accrued when one team's jammer makes her second, and subsequent, pass through the pack of blockers, in effect lapping the pack. Activity occurs in intervals called "jams," and a single jam lasts for a maximum of 2 minutes. Flat track roller derby is a contact sport; blockers are allowed to initiate contact with another player to compete for track position using any of the following body parts: upper arm (shoulder to elbow), torso, hips, "booty," and mid to upper thigh. Roller derby tournaments often involve multiple pairwise bouts in a single day between several teams, one home team and multiple visiting teams from different geographical locations. Players within a team practice together on a regular basis, and thus come into frequent physical contact, and live in or near the same city. Teams involved in this study were from Eugene, OR (Emerald City Roller Girls); Washington, DC (DC Roller Girls) and San Jose, CA (Silicon Valley Roller Girls).

PeerJ PrePrints

Ethics Statement

Written consent forms were signed and collected from all participating subjects. The Institutional Review Board Initial Application Form for the study was reviewed and approved by the University of Oregon IRB with the Office for Protection of Human Subjects in January 2012 (protocol #10262011.038). The Willamalane Park and Recreation District Human Resources office granted written permission for the study to take place in their recreation facility. Written permission was acquired from the three teams coaches and administrators.

Sample Collection

Microbial communities inhabiting skin vary greatly across the human body (Grice *et al.* 2009; 2008; Human Microbiome Project Consortium 2012). We chose the upper arm as our focal skin sample site. The upper arm is the one skin region on roller derby skaters that is nearly universally exposed and frequently contacted during a bout. All samples were collected Feb. 10, 2012, at the "Big O" Tournament in Eugene, OR, USA. The two bouts that were sampled took place at 12:00pm (Emerald City vs. Silicon Valley) and 6:00pm (Emerald City vs. DC). DC had already played in one bout the same day at 10:00am, but Emerald City and Silicon Valley had not played that day prior to bout 1. Samples were collected by swabbing individual's upper arms in a c. 4 cm by 5 cm area of skin with nylon-flocked swabs (COPAN Flock Technologies, Brescia, Italy). Both arms were swabbed on each player at each sampling point. Samples were stored at -20° C until DNA extraction. Total number of jams was recored for each player, and multiplied by 2 minutes (maximum jam length) to approximate total time played per person. Four swab samples were also taken from the floor of the facility (track) following the tournament using the same swabbing method and surface area as the arm samples.

DNA Extraction, amplification and sequencing

Whole genomic DNA was extracted using the MO BIO PowerWater DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA) according to manufacturers instructions with the following modifications: swab tips were incubated with Solution PW1 in a 65° C water bath for 15 minutes prior to bead beating; bead beating length was extended to 10 minutes; and samples were eluted in 50 μL Solution PW6. Dual-arm samples from each player were combined for DNA extraction.

PeerJ PrePrints

A region of the V4 region of the 16S rRNA gene was amplified using a modified F515/R806 primer combination (5′-GTGCCAGCMGCCGCGGTAA-3′, 5′-TACNVGGGTATCTAATCC-3′) (Caporaso *et al.* 2011b; Claesson *et al.* 2010). Amplification proceeded in two steps using a custom Illumina preparation protocol, where PCR1 was performed with forward primers that contained partial unique barcodes and partial Illumina adapters. The remaining ends of the Illumina adapters were attached during PCR2, and barcodes were recombined *in silico* using paired-end reads. Adapter sequences are detailed in supplemental materials. All extracted samples were amplified in triplicate for PCR1 and triplicates were pooled before PCR2. PCR1 (25 μL total volume per reaction) consisted of the following steps: 5 μL GC buffer (Thermo Fisher Scientific, U.S.A.), 0.5 μL dNTPs (10mM, Invitrogen), 0.25 μL Phusion Hotstart II polymerase (Thermo Fisher Scientific, U.S.A.), 13.25 μL certified nucleic-acid free water, 0.5 μL forward primer, 0.5 μL reverse primer, and 5 μL template DNA. The PCR1 conditions were as follows: initial denaturation for 2 minutes at 98° C; 22 cycles of 20 seconds at 98° C, 30 seconds at 50° C and 20 seconds at 72° C; and 72° C for 2 minutes for final extension. After PCR1, the triplicate reactions were pooled and cleaned with the QIAGEN Minelute PCR Purification Kit according to the manufacturers protocol (QIAGEN, Germantown, MD). Ten μL of 3M NaOAc (pH 5.2) was added to decrease the pH of the pooled reactions and facilitate efficient binding to the spin column during cleanup. Samples were eluted in 11.5 μL of Buffer EB. For PCR2, a single primer pair was used to add the remaining Illumina adaptor segments to the ends of the concentrated amplicons of PCR1. The PCR2 (25 μL volume per reaction) consisted of the same combination of reagents that was used in PCR1, along with 5 μL concentrated PCR1 product as template. The PCR 2 conditions were as follows: 2 minutes denaturation at 98° C; 12 cycles of 20 seconds at 98° C, 30 seconds at 66° C and 20 seconds at 72° C; and 2 minutes at 72° C for final extension. Amplicons were size-selected by gel electrophoresis: gel bands at c. 440bp were extracted and concentrated, using the ZR-96 Zymoclean Gel DNA Recovery Kit (ZYMO Research, Irvine, CA), following manufacturer's instructions, quantified using a Qubit Fluoromoeter (Invitrogen, NY), and pooled in equimolar concentrations for library preparation for sequencing. Samples were sent to the Georgia Genomics Facility at the University of Georgia (Athens, GA; www.dna.uga.edu), and sequenced on the Illumina MiSeq platform as paired-end reads. Duplicate MiSeq runs, each including all samples, were combined prior to sequence processing.

PeerJ PrePrints

Sequence Processing

Raw sequences were processed using the FastX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit) and the QIIME pipeline (Caporaso *et al.* 2010). Barcodes were recombined from paired-end reads, but only the forward read was used for analysis due to low quality in the reverse reads. All sequences were trimmed to 112 bp, including a 12 bp barcode, and low quality sequences were removed. Quality filtering settings were as follows: minimum 30 quality score over at least 75% of the sequence read; no ambiguous bases allowed; 1 primer mismatch allowed. After quality control and barcode assignment, the remaining 1,368,938 sequences were binned into operational taxonomic units (OTUs) at a 97% sequence similarity cutoff using uclust (Edgar 2010). The highest-quality sequences from each OTU cluster were taxonomically identified using BLAST reference sequences from Greengenes (DeSantis *et al.* 2006). Plant-chloroplast and mitochondrial OTUs were removed. Not all samples returned the same number of sequences. Following rarefaction precedents (e.g., Human Microbiome Project Consortium 2012; Kuczynski *et al.* 2010) we rarefied all samples to 500 sequences per sample. Samples with less than 500 sequences were not used in subsequent analyses (Table 1). Three of the track samples contained enough sequences to be considered in analysis, and were processed exactly as the rest of the samples, but were not used in any ordination analysis. Sequence files and metadata for all samples used in this study have been deposited in MG-RAST (ID 4506457.3 – 4506498.3).

Statistical Analysis

All statistical analyses were performed in `R`. Community variation among samples, or β-diversity, was calculated using the quantitative, taxonomy-based Canberra distance, implemented in the `vegan` package (Oksanen *et al.* 2011). Non-metric multidimensional scaling (NMDS) was performed using the `bestnmds` function in the `labdsv` package (Roberts 2010), using 20 random starts. Discriminant analysis of within-group similarity was conducted using permutational MANOVA with the `adonis` function in `vegan`. To determine whether skin microbial communities became more similar to one another after playing in a bout, we used a β-dispersion test with the `betadisper` function in `vegan`. This test is a multivariate analog of Levene's test for homogeneity of variances (Anderson *et al.* 2006), and it tests for a significant difference in sample heterogeneity between groups (i.e. the spread of data points in ordination space). The relationship between time played and change in community composition was assessed with Pearson's

PeerJ PrePrints

correlation test by comparing individual players' pairwise community distances with their estimated cumulative times during a bout.

Results

Illumina sequencing of the V4 region of the 16S rRNA genes produced 1,368,938 barcoded sequences. After quality filtering and rarefaction, 82 samples were considered during analysis, taken from 3 teams and two bouts (Table 1). Emerald City Roller Girls (EC) played in both bouts and thus were included twice in the analyses as two different team groups; Silicon Roller Girls (SI) and DC Roller Girls (DC) played in the first and second bouts, respectively, against EC. Including EC players twice in the study allowed us to evaluate the change in community composition in a single team after playing successive bouts. Rarefaction to 500 sequences per sample left 1034 bacterial OTUs, with the most abundant OTU (*Corynebacterium* sp.) representing c. 34% of total sequences, and c. 55% of all OTUs represented by a single sequence. The bacterial taxa identified in our skin samples were consistent with what has been reported in other skin microbiome studies (e.g., Caporaso *et al.* 2011a; Costello *et al.* 2009; Grice & Segre 2011; Kong 2011) When considered at the taxonomic class level, the majority of sequences were *Actinobacteria* (57.9%), followed by *Bacilli* (23.4%), *Gammaproteobacteria* (7.4%), *Betaproteobacteria* (3.7%), *Alphaproteobacteria* (2.7%), and *Clostridia* (1.3%). All skin samples were dominated by skin-associated genera (especially *Corynebacterium*, *Micrococcus*, *Staphylococcus*, and *Acinetobacter*), and by oral-associated genera (including *Neisseria* and *Rothia*), both before and after bouting. Post-bout samples, however, did contain higher relative abundances of a few soil- and plant-associated genera, especially *Arthrobacter* and *Xanthomonas*. Normalized OTU richness, at the 500 sequences per sample level (mean richness = 67.6 OTUs), was not significantly different after a bout ($t = 0.007$; $p = 0.9$; from a Welch two-sample t-test), and neither was Shannon-Wiener Diversity ($t = 0.11$; $p = 0.9$; from a Welch two-sample t-test; mean = 2.66).

Were players' skin microbiomes predicted by team membership? Bacterial communities detected on players upper arms from different teams were significantly different before playing a bout, as well as after playing a bout (Table 2). In other words, the skin microbiome of an individual player was predicted by team membership. Teams clustered together in ordination space using a non-metric multidimensional scaling representation of players' skin microbiomes both before (Figure 1a) and after

PeerJ PrePrints

(Figure 1b) playing a bout, based on Canberra taxonomic distances. Though team clustering is significant in both cases (before and after a bout), there is a greater degree of overlap between the teams following bouts.

The home team's pre-bout bacterial communities (EC) were more similar on average to communities detected on the track than the two visiting teams (p <0.001; from a Welch two-sample t-test; Figure 2). All players were more dissimilar on average from track samples (mean Canberra distance = 0.89) than from all other players (mean Canberra distance = 0.83; p <0.001; from a Welch two-sample t-test). Player bacterial communities did not become more similar to the track after a bout, and in fact both bout 2 teams (EC & DC) became less similar following a bout (p = 0.008 & 0.003, respectively; from Welch two-sample t-tests).

Were team-specific skin microbiomes different after playing a bout? When teams were considered separately, bacterial communities detected on players upper arms before a bout were significantly different than those detected after the bout in all cases (Table 2; Figure 3). We also detected a signal of already having played in a bout. Two teams, Emerald City and DC, had already played in a bout the morning of the tournament, and that was a significant predictor of community composition before the second bout (F-statistic = 2.16; p-value < 0.001; EC ▲ & DC ▲ in Figure 1a).

Did opposing teams' skin microbiomes become more similar after competing in a bout? All players' skin microbiomes were more similar to one another after competing in a bout. To test this we conducted a β-dispersion test, which compared all players before and after bouting. A significant reduction in β-dispersion between groups (before vs. after) confirmed that communities became more similar (based on Canberra distances; F = 11.79; p < 0.001; Table 3; Figure 4). When each team was considered separately, both teams in bout 1 experienced a significant β-dispersion reduction as did EC after playing in bout2, while DC did not (Table 3; Figure 4). Both teams in bout 2 had already played a bout previously in the day; neither team in bout 1 had played during the same day. Changes in bacterial communities before and after a bout were not correlated with each players time spent in a bout (Pearsons correlation test; ρ = 0.12; p = 0.45).

PeerJ PrePrints

Discussion

Bacteria are ubiquitous. Those inhabiting the human body have received increased attention in recent years, owing to a greater appreciation of the interrelated nature of humans and their microbiome, an improved understanding of microbial ecology, and an unprecedented ability to detect fine-scale microbial community changes with high-throughput sequencing technology (Human Microbiome Project Consortium 2012). The skin is the largest organ and an important barrier that regulates microbial entry into the human body. Despite the importance of the skin ecosystem to human health and well-being, we know very little about the forces that shape microbial structure and composition in the skin environment. The present study was designed as a way to understand how human to human contact influences the skin microbiome, since contact has long been acknowledged as a major dispersal vector for skin bacterial communities (Hamburger Jr. 1947; Pittet *et al.* 2006).

We found that team membership was a strong predictor of the skin microbiome. This could be because the three teams surveyed were from three distinctly different geographic locations within the United States (Eugene, OR; San Jose, CA; and Washington, DC), each associated with a different climate, urban setting, and outdoor macrobiota. These cities may also have very different environmental microbiota. Blaser *et al.* (2012) recently found that human populations from different geographical locations share distinct skin microbial communities. Consistent with this hypothesis, we found that home team (EC) microbiomes were more similar to their home track than either of the visiting teams prior to bouting. Since this is also the EC practice track, it is perhaps unsurprising that EC players share some of their microbiome with the track surface since they shed skin cells and frequently come into direct contact the floor. While a variety of factors likely contribute to this geographic signature, it is plausible that human contact plays a role. In our study, athletes on the same team likely share portions of their skin microbiomes during the many hours of practice every week. All teams appeared to retain their 'microbial fingerprint' even after playing a series of bouts.

Although teams retained their microbial fingerprint, we found that team microbial communities became overall more similar to one another after players competed in a bout. This was found both when considering all players together and when considering each team individually, though the latter was not the case for DC, who played in the second bout. Several reasonable explanations arise given these results: 1) players were coming into repeated physical contact with their teammates and those from opposing

PeerJ PrePrints

teams, often using the sampled area of their upper arms, and potentially sharing portions of their skin microbiomes; 2) players were acquiring microbial transients from the built environment, since roller derby and crowd movements likely stirred up dust from the recreational venue, and players also frequently fall on the floor; and 3) all players were exercising, and exercise produces predictable changes in skin habitat conditions that are likely to affect bacterial communities. Although humans have been estimated to contribute more than 10^6 airborne microbial cells per-hour (Qian *et al.* 2012), culture-based disease transmission studies suggest that direct contact with humans and other surfaces is a stronger bacterial dispersal vector than airborne bodily dispersal (Casewell & Phillips 1977; Pessoa-Silva *et al.* 2004; Pittet *et al.* 2006). In our study we found that human to track surface contact did not seem to explain the observed shifts in community composition, since none of the four team groups became more similar to the track samples after playing in a bout. Together, this leaves the first of these potential mechanisms as a more parsimonious explanation than the second. The current study was not set up to conclusively rule out the potential for exercise-related bodily changes to alter skin bacterial communities, though it does seem unlikely that 60 minutes of elevated skin temperature and perspiration would be long enough for microbial growth dynamics to effect the magnitude of changes observed, given that bacterial doubling times generally exceed 20 minutes even in optimal conditions. It is possible that exercise results in migration from subcutaneous habitats to the skin surface, but little is known about this potential mechanism. Our results can likely be attributed to both dispersal between humans and from the built environment, and athletic activities (change in pH, temperature and moisture at the skin surface), and future research into these two drivers can help to understand the controls on the human skin microbiome.

We know very little about how our social, family, and professional interactions shape our microbial identities. Contact sports are an ideal setting in which to study how human to human interactions influence our microbial ecosystems. As the rise of mega-cities and population growth continues, humans may experience an increased rate of person to person contact mediated by urban living and global travel. To predict the implications of these changes will require, in part, understanding the ecological and evolutionary forces that act on the skin microbiome. A thorough comprehension of the drivers of the skin microbiome is still emerging; novel approaches to studying our skin ecosystems will likely have lasting implications for health care, disease transmission, and our understanding of urban environment microbiology.

PeerJ PrePrints

PeerJ PrePrints

Acknowledgments

This study was funded in part by a grant from the Alfred P. Sloan Foundation to the Biology and the Built Environment Center, University of Oregon. We would like to thank the players and coaches who facilitated and participated in this study; we are particularly grateful to Burnadeth, Katarina Van Rotten, Rex Havoc, Vexine, Blue Ruin, and Agent Orange. Willamalane Park and Recreation District Human Resources office provided permission to conduct sampling in the recreation facility. H. Arnold, R. Mueller, P. Pillai, J. Reichman, Z. Stephens, A. Womack, M. Naidoo, and Super Cake helped with sampling and development of molecular protocols.

References

Anderson MJ, Ellingsen KE, McArdle BH. 2006. Multivariate dispersion as a measure of beta diversity. *Ecology Letters* 9: 683–693.

Blaser MJ, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Estrada I, Gao Z, Clemente JC, Costello EK, Knight R. 2012. Distinct cutaneous bacterial assemblages in a sampling of south american amerindians and us residents. *ISME Journal* .

Boyce JM, Potter-Bynoe G, Chenevert C, King T. 1997. Environmental contamination due to methicillin-resistant *Staphylococcus aureus*: possible infection control implications. *Infection Control and Hospital Epidemiology* 18: 622–627.

Capone KA, Dowd SE, Stamatas GN, Nikolovski J. 2011. Diversity of the human skin microbiome early in life. *Journal of Investigative Dermatology* 131: 2026–2032.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Tumbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7: 335–336.

Caporaso JG, Lauber CL, Costello EK, Berg-Lyons D, Gonzalez A, Stombaugh J, Knights D, Gajer P,

276 Ravel J, Fierer N, Gordon JI, Knight R. 2011a. Moving pictures of the human microbiome. *Genome*
277 *Biology* 12: R50, ISSN 14656914.

278 Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight
279 R. 2011b. Global patterns of 16s rRNA diversity at a depth of millions of sequences per sample. *PNAS*
280 108: 4516–22.

281 Casewell M, Phillips I. 1977. Hands as route of transmission for *Klebsiella* species. *British Medical Journal*
282 2: 1315–1317.

283 Claesson MJ, Wang Q, O'Sullivan O, Greene-Diniz R, Cole JR, Ross RP, O'Toole PW. 2010. Comparison
284 of two next-generation sequencing technologies for resolving highly complex microbiota composition
285 using tandem variable 16s rRNA gene regions. *Nucleic Acids Research* 38: e200.

286 Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. 2009. Bacterial community variation
287 in human body habitats across space and time. *Science* 326: 1694–1697.

288 Davis MF, Iverson SA, Baron P, Vasse A, Silbergeld EK, Lautenbach E, Morris DO. 2012. Household
289 transmission of meticillin-resistant *staphylococcus aureus* and other staphylococci. *Lancet Infectious*
290 *Diseases* 12: 703–716.

291 DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen
292 GL. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with
293 ARB. *Applied and Environmental Microbiology* 72: 5069–72.

294 Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, Knight R. 2010.
295 Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body
296 habitats in newborns. *PNAS* 107: 11971–11975.

297 Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26:
298 2460–2461.

299 Fierer N, Hamady M, Lauber CL, Knight R. 2008. The influence of sex, handedness, and washing on the
300 diversity of hand surface bacteria. *PNAS* 105: 17994–17999.

PeerJ PrePrints

Fujimura KE, Johnson CC, Ownby DR, Cox MJ, Brodie EL, Havstad SL, Zoratti EM, Woodcroft KJ, Bobbitt KR, Wegienka G, Boushey HA, Lynch SV. 2010. Man's best friend? the effect of pet ownership on house dust microbial communities. *Journal of Allergy and Clinical Immunology* 126: 410–412.

Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, Bouffard GG, Blakesley RW, Murray PR, Green ED, Turner ML, Segre JA. 2009. Topographical and temporal diversity of the human skin microbiome. *Science* 324: 1190–1192.

Grice EA, Kong HH, Renaud G, Young AC, Bouffard GG, Blakesley RW, Wolfsberg TG, Turner ML, Segre JA. 2008. A diversity profile of the human skin microbiota. *Genome Research* 18: 1043–1050.

Grice EA, Segre JA. 2011. The skin microbiome. *Nature Reviews Microbiology* 9: 244–253.

Hamburger Jr M. 1947. Transfer of beta hemolytic streptococci by shaking hands. *American Journal of Medicine* 2: 23–25.

Hospodsky D, Qian J, Nazaroff WW, Yamamoto N, Bibby K, Rismani-Yazdi H, Peccia J. 2012. Human occupancy as a source of indoor airborne bacteria. *PLoS One* 7: e34867.

Human Microbiome Project Consortium. 2012. Structure, function and diversity of the healthy human microbiome. *Nature* 486: 207–14.

Kembel SW, Jones E, Kline J, Northcutt D, Stenson J, Womack AM, Bohannan BJ, Brown GZ, Green JL. 2012. Architectural design influences the diversity and structure of the built environment microbiome. *The ISME Journal* 1–11, ISSN 17517362.

Kong HH. 2011. Skin microbiome: genomics-based insights into the diversity and role of skin microbes. *Trends in Molecular Medicine* 17: 320–328.

Kuczynski J, Liu ZZ, Lozupone C, McDonald D, Fierer N, Knight R. 2010. Microbial community resemblance methods differ in their ability to detect biologically relevant patterns. *Nature Methods* 7: 813–821.

Noble WC, Habbema JDF, van Furth R, Smith I, de Raay C. 1976. Quantitative studies on the dispersal of skin bacteria into the air. *Journal of Medical Microbiology* 9: 53–61.

PeerJ PrePrints

Oksanen J, Blanchet FG, Kindt R, Legendre P, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2011. *vegan: Community Ecology Package*. URL `http://CRAN.R-project.org/package=vegan`, R package version 1.17-6.

Pessoa-Silva CL, Dharan S, Hugonnet S, Touveneau S, Posfay-Barbe K, Pfister R, Pittet D. 2004. Dynamics of bacterial hand contamination during routine neonatal care. *Infection Control and Hospital Epidemiology* 25: 192–197.

Pittet D, Allegranzi B, Sax H, Dharan S, Pessoa-Silva CL, Donaldson L, Boyce JM, WHO Global Patient Safety Challenge, World Alliance for Patient Safety. 2006. Evidence-based model for hand transmission during patient care and the role of improved practices. *Lancet Infectious Diseases* 6: 641–52.

Qian J, Hospodsky D, Yamamoto N, Nazaroff WW, Peccia J. 2012. Size-resolved emission rates of airborne bacteria and fungi in an occupied classroom. *Indoor Air* 22: 339–51.

Roberts DW. 2010. *labdsv: Ordination and Multivariate Analysis for Ecology*. URL `http://CRAN.R-project.org/package=labdsv`, R package version 1.4-1.

PeerJ PrePrints

Figure Legends

Figure 1. Variation in skin microbial community composition is significantly explained by team identity. Ordination diagrams (axes 1 and 2 from separate 3-dimensional NMDS ordinations) summarizing similarity of skin bacterial community composition of all players. A) Points represent players before bout 1 (EC ● vs. SI ●) and before bout 2 (EC ▲ vs. DC ▲). Corresponding-colored ellipses show standard deviations around community variances from each team. The skin bacterial communities of the four team groups were significantly different before playing a bout ($p < 0.001$; from permutational MANOVA on Canberra taxonomic distances). B) The four team groups are also significantly different after playing bouts ($p < 0.001$), though more overlap is observed between teams after bout 1 (EC ○ vs. SI ○) and after bout 2 (EC △ vs. DC △). NMDS 3-dimensional stress = 19.66 (A) & 17.55 (B).

Figure 2. Home team (EC) players' skin microbiomes were more similar to the microbial community detected on the roller derby track than visiting teams. When each player's pre-bout skin microbiomes were compared to the microbial communities found on the track surface, **Emerald City** players skin microbiomes were significantly more similar on average to the three track samples than were the skin microbiomes of players from **Silicon Valley** or **DC**.

Figure 3. Team-specific micobiomes are significantly different after playing in a bout. NMDS ordination diagrams summarizing similarity of skin bacterial community composition when all players are compared within their own teams before and after a bout. All ordinations are based on Canberra taxonomic distances. A) Emerald City before ● and after ○ bout 1; B) Silicon Valley before ● and after ○ bout 1; C) Emerald City before ▲ and after △ bout 2; D) DC before ▲ and after △ bout 2. Corresponding-colored ellipses are standard deviations on community variances for each group. All teams showed significantly different microbial communities before vs. after a bout. NMDS 3-dimensional stress: A=8.1, B=10.47, C=16.2, D=17.65.

Figure 4. Bacterial community variance is reduced after playing in a bout for all players and for three of the four teams individually. When all players were considered, regardless of team identity, bacterial communities were significantly more similar to one another after a bout than they were before a bout ($p < 0.001$). Both teams in bout 1 (EC and SI), as well as EC in bout 2, showed the same microbial community convergence. Points are jittered around the x-axis to more clearly describe distributions. All p-values are from β-dispersion tests; a lower mean community variance for the "after-bout" points means that players' skin micobiomes were more similar to one another after playing in a bout. Colored points correspond to Table 1 and Figures 1 and 3.

PeerJ PrePrints

Figure 1

Figure 1

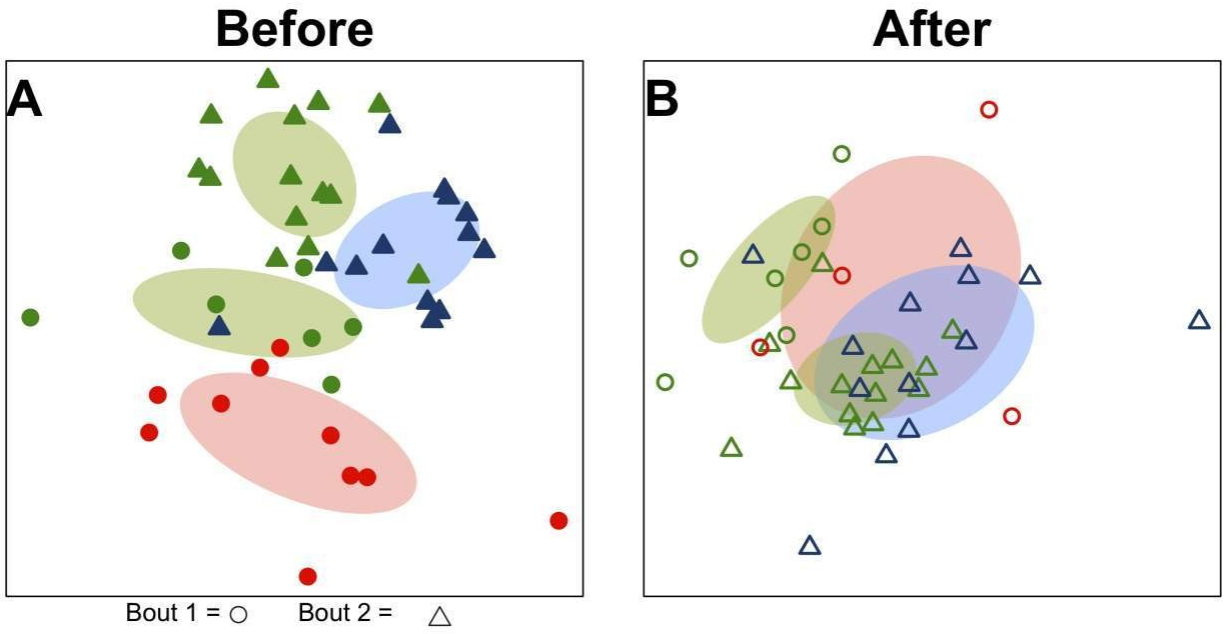

Figure 1. Variation in skin microbial community composition is significantly explained by team identity. Ordination diagrams (axes 1 and 2 from separate 3-dimensional NMDS ordinations) summarizing similarity of skin bacterial community composition of all players. A) Points represent players before bout 1 (EC ● vs. SI ●) and before bout 2 (EC ▲ vs. DC ▲). Corresponding-colored ellipses show standard deviations around community variances from each team. The skin bacterial communities of the four team groups were significantly different before playing a bout ($p < 0.001$; from permutational MANOVA on Canberra taxonomic distances). B) The four team groups are also significantly different after playing bouts ($p < 0.001$), though more overlap is observed between teams after bout 1 (EC ○ vs. SI ○) and after bout 2 (EC △ vs. DC △). NMDS 3-dimensional stress = 19.66 (A) & 17.55 (B).

PeerJ PrePrints

Figure 2

Figure 2

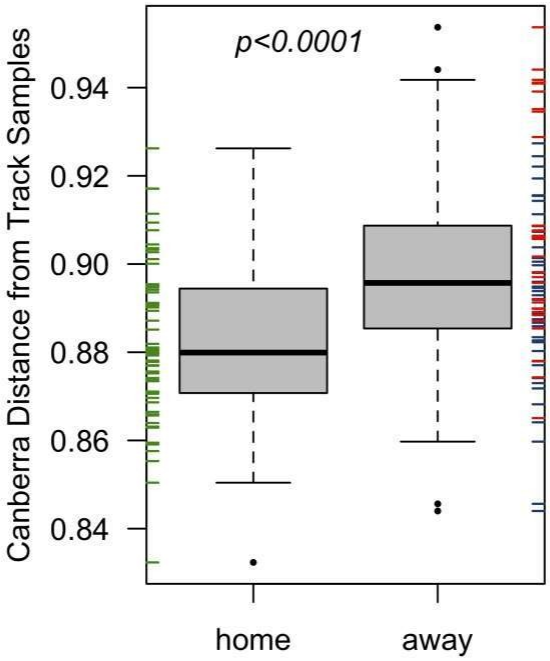

Figure 2. Home team (EC) players' skin microbiomes were more similar to the microbial community detected on the roller derby track than visiting teams. When each player's pre-bout skin microbiomes were compared to the microbial communities found on the track surface, **Emerald City** players skin microbiomes were significantly more similar on average to the three track samples than were the skin microbiomes of players from **Silicon Valley** or **DC**.

PeerJ PrePrints

Figure 3

Figure 3

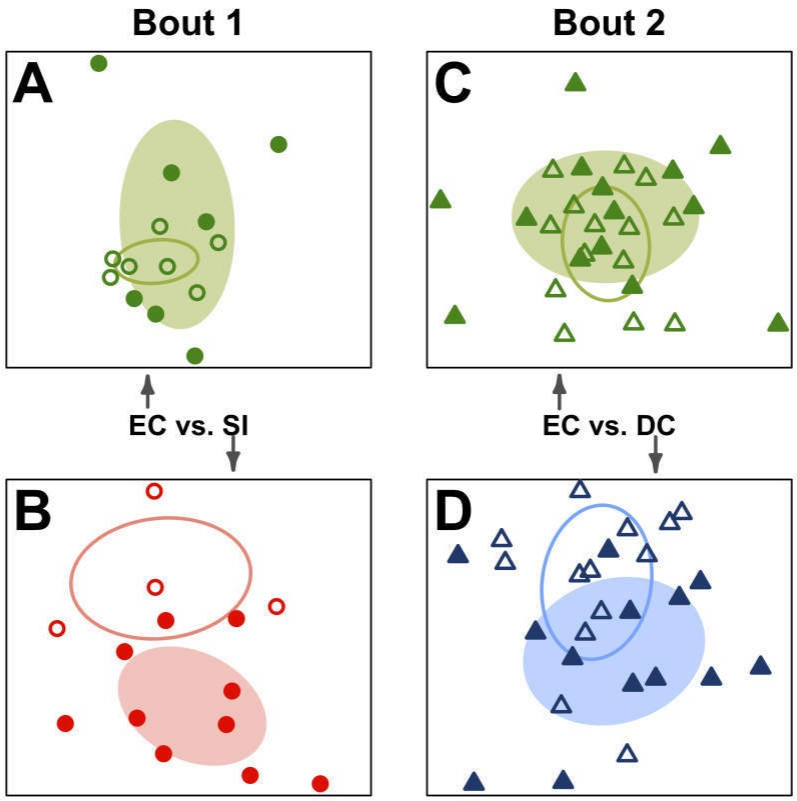

Figure 3. Team-specific micobiomes are significantly different after playing in a bout. NMDS ordination diagrams summarizing similarity of skin bacterial community composition when all players are compared within their own teams before and after a bout. All ordinations are based on Canberra taxonomic distances. A) Emerald City before ● and after ○ bout 1; B) Silicon Valley before ● and after ○ bout 1; C) Emerald City before ▲ and after △ bout 2; D) DC before ▲ and after △ bout 2. Corresponding-colored ellipses are standard deviations on community variances for each group. All teams showed significantly different microbial communities before vs. after a bout. NMDS 3-dimensional stress: A=8.1, B=10.47, C=16.2, D=17.65.

PeerJ PrePrints

Figure 4

Figure 4

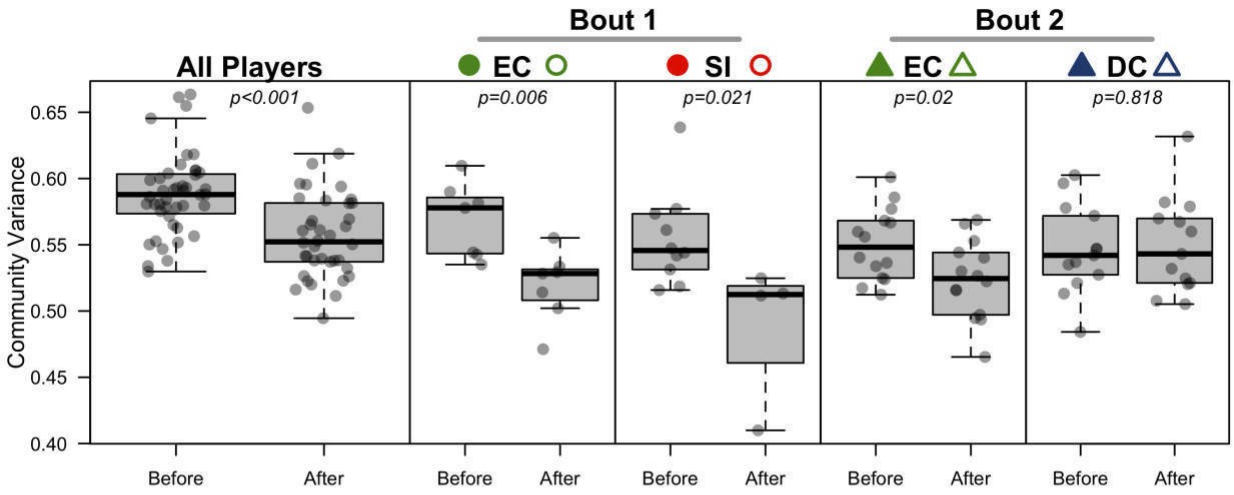

Figure 4. Bacterial community variance is reduced after playing in a bout for all players and for three of the four teams individually. When all players were considered, regardless of team identity, bacterial communities were significantly more similar to one another after a bout than they were before a bout ($p < 0.001$). Both teams in bout 1 (EC and SI), as well as EC in bout 2, showed the same microbial community convergence. Points are jittered around the x-axis to more clearly describe distributions. All p-values are from β-dispersion tests; a lower mean community variance for the "after-bout" points means that players' skin micobiomes were more similar to one another after playing in a bout. Colored points correspond to Table 1 and Figures 1 and 3.

PeerJ PrePrints

Table 1(on next page)

Table 1

PeerJ PrePrints

Table 1. Description of the two roller derby bouts considered in analyses.

Team	n Players Before	n Players After	Bout	1^{st} Bout of the Day
Emerald City	● 7	○ 7	1	yes
Silicon Valley	● 10	○ 4	1	yes
Emerald City	▲ 14	△ 14	2	no
DC	▲ 13	△ 13	2	no

PeerJ PrePrints

Two different bouts were sampled; bout 2 occurred approximately 5 hours after bout 1. Emerald City Roller Girls played in both bouts. Neither team in bout 1 had played a bout previously in the day, but both teams in bout 2 had done so. Total skin samples considered in analysis = 82. Colored points correspond to those used in all figures.

Table 2(on next page)

Table 2

PeerJ PrePrints

Table 2. Results from Permutational MANOVA on Canberra distances among skin bacterial communities sampled from players before and after bouting.

Comparison	Team	DF_{resid}	F-statistic	p-value	Bout
Before/After	Emerald City	12	1.25	0.017∗	1
	Silicon Valley	12	1.39	0.005∗	1
	Emerald City	26	1.22	0.011∗	2
	DC	24	1.35	<0.001∗	2
	all players	80	1.96	<0.001∗	–
Before	all teams	40	1.74	<0.001∗	–
After	all teams	34	1.27	<0.001∗	–

∗ Significant at $p<0.05$ level.

PeerJ PrePrints

Each team was considered individually when testing for intra-team before/after community shifts, while teams were considered together for the "all players" before/after test. Team identity was used as a grouping factor to test inter-team clustering ("all teams"), both before and after bouts. Emerald City was considered to be two different teams (bout 1 and bout 2) in analyses.

Table 3(on next page)

Table 3

PeerJ PrePrints

Table 3. Results from β-dispersion ANOVA on Canberra distances when comparing community variances from each team before and after, as well as all players regardless of team identity.

Team	DF_{resid}	F-statistic	p-value	Bout
Emerald City	12	11.34	0.006∗	1
Silicon Valley	12	7.16	0.02∗	1
Emerald City	26	6.03	0.02∗	2
DC	24	0.05	0.82	2
all players	80	19.07	<0.001	–

∗ Significant at $p<0.05$ level.

The first four tests describe β-dispersion tests (comparison of within-team bacterial community variance) when each team is considered individually before and after a bout, and the fifth ignores team identity. Results indicate that skin bacterial communities from Emerald City (bout 1) and Silicon Valley players both became more similar following a bout, as did Emerald City from their 2nd bout. But this was not the case for DC after playing in bout 2. Bacterial communities became more similar when all players were considered in the same analysis.