Health Sciences Research
2018; 5(2): 43-49
http://www.aascit.org/journal/hsr
ISSN: 2375-379X

Clean Hands → Healthy Wrestlers: Effectiveness of Hand Cleaning in Reducing Bacterial Load During Wrestling Competitions

Linda Mull Young[1], Brooke Peters[1], Addie Davis[1], Katherine Krynak[1], Ronald Beaschler[2], Holly Dyer[1], Vicki Abrams Motz[1, *]

[1]Department of Biological and Allied Health Sciences, Ohio Northern University, Ada, Ohio
[2]Department of Athletics, Ohio Northern University, Ada, Ohio

Email address

v-motz@onu.edu (V. A. Motz)
[*]Corresponding author

Citation

Linda Mull Young, Brooke Peters, Addie Davis, Katherine Krynak, Ronald Beaschler, Holly Dyer, Vicki Abrams Motz. Clean Hands → Healthy Wrestlers: Effectiveness of Hand Cleaning in Reducing Bacterial Load During Wrestling Competitions. *Health Sciences Research.* Vol. 5, No. 2, 2018, pp. 43-49.

Received: January 30, 2018; **Accepted**: March 7, 2018; **Published**: May 16, 2018

Abstract: The spread of infections due to close contact with other grapplers, mats and apparel has been of major concern in the sport of wrestling. This study compared the use of a variety of hand and mat cleaners and application methods to determine their efficacy in reducing bacterial load in the wrestling environment. Effect of cleanser type was compared in wrestling practices, laboratory experiments with controlled application of bacteria, and in one large-scale, multi-school invitational meet. Repeated use of non-residual cleaners such as cleaning mats with bleach and hands with alcohol gel or single use of residual hand cleansers such as Clean Contact™ were most effective at reducing bacterial abundance and resulted in similar bacterial community composition on wrestling mats. Based on this data, judicious hand sanitizing has the potential to protect wrestlers from bacterial contamination.

Keywords: Hand Cleansing, Sports Infections, Pathogen Transmission, Microbiome

1. Introduction

The transmission of skin infections between wrestlers has been documented since the first National Collegiate Athletic Association (NCAA) men's wrestling championship held in 1928. [1] This problem continues today, at practices, as well as competitive meets [2], both by skin to skin contact [3] or through fomites such as wrestling mats [4]. Hand washing prior to grappling reduces pathogens on hands presumably decreasing transmission of infection. [5] The current study identifies the best active component and application method (wipes/gel/foam/spray) to prevent spread of infection by reducing bacterial load on wrestlers. Mechanisms of action associated with the products used included: protein denaturation by oxidizing agents (bleach), heavy metal antiseptics (colloidal silver); cell dehydration by alcohols (ethanol, methanol); and plasma membrane disruption by phenolic compounds (thymol, eucalyptol), detergents (sodium laureth sulfate), quaternary ammonium compounds (benzalkonium chloride), and chlorhexidine gluconate. The latter two claim to have residual activity, such that they continue to kill bacteria beyond the time of application. Because it has been shown that increased bacterial load on mats increases load on wrestlers [5, 6], a component of this investigation determined whether hand washing altered the bacterial community (microbiome) on the mats and if the effects were cleaner specific.

2. Methods

Hand cleansers come in many forms. Studies compared effectiveness of chemical composition and application method for products which are marketed to wrestling coaches (Table 1). Use of cleansers and protocols were IRB approved.

Table 1. *Hand cleansers evaluated in this study.*

Code	Delivery Method	Source	Active Compounds	Application Method
AG	Gel	Members Mark Sam's Club	62% Ethyl Alcohol	One pump squirt into hand-rub hands together another squirt on one hand-rub back of neck Practice: repeat every 15 min Meet: repeat before each bout
AW	Wipe	Medistaph Antimicrobial Skin Wipes	66.5% Ethyl Alcohol	Student was handed a wipe and told to cleanse hands-given a second wipe and told to cleanse back of neck
AECS	Spray	Battleskin Spray Battleskin LLC	Ethanol Methanol Eucalyptol Chlorhexidine Digluconate	One spritz into hand -rub hands together-another spritz on one hand-rub back of neck Battleskin hand treatment repeated after three hours
BF	Foam	Clean Contact Woodbine Products	0.1% Benzalkonium Chloride	One pump squirt into hand-rub hands together another squirt on one hand-rub back of neck
BW	Wipe	Wipes Plus Progressive Products LLC	0.13% Benzalkonium Chloride	Student was handed a wipe and told to cleanse hands-given a second wipe and told to cleanse back of neck
CG	Wash	Hibiclens	4% W/V Chlorhexidine Gluconate	Wet hands from tap-one squirt from dispenser- rub hands together-rinse with tap water-dry on paper towel
CSF	Foam	Theraworx Technology Avadin Tech Inc	Colloidal Silver Cocamidopropyl Betain	One spray onto paper towel used to wash one hand-transfer towel to other hand-spray again to wash second hand-spray again to cleanse back of neck
ETG	Gel	Defense Gel Defense Soap LLC	Eucalyptol Tea Tree Oil	One pump squirt into hand-rub hands together-another squirt on one hand-rub back of neck
ETW	Wipe	Defense Wipe Defense Soap LLC	1% Eucalyptol 1% Tea Tree Oil	Student was handed a wipe and told to cleanse hands-given a second wipe and told to cleanse back of neck
SL	Wash	Episoft Soap Ecolab Inc Professional Products Div	Sodium Laureth Sulfate Cocamidopropyl Betain	Wet hands from tap-one squirt from dispenser-rub hands together-rinse with tap water-dry on paper towel
TAS	Spray	Formulated in our lab	0.05% Thyme Oil Solution In A 5% Aqueous Aloe Suspension	One spritz into hand-rub hands together-another spritz on one hand-rub back of neck
TAW	Wipe	Formulated in our lab	TAG on alcohol wipe with alcohol evaporated off	Student was handed a wipe and told to cleanse hands-given a second wipe and told to cleanse back of neck
TS	Spray	Benefect Natural Hand Sanitizer Life Products	0.05% Thymol (*Thymus vulgaris* oil)	One spritz into hand-rub hands together-another spritz on one hand-rub back of neck

2.1. Controlled Studies – Wrestling Practice

To examine types of hand cleaners, twenty-two collegiate wrestlers were divided into four weight divisions, each of which cleaned their hands with a different cleaner (TAS, BF, CG or AG). Wrestlers practiced for 1.5 hours and hands were swabbed using a rayon swab (all rayon swabs were moistened with Stuart's medium). To determine the impact of application method, the right and left hands of twenty collegiate wrestlers and the backs of their necks (which is a site of most frequent contact during grappling) were swabbed using a rayon swab prior to practice (see swab processing protocol below in section 2.4). Then the right hand was cleaned by a volunteer wearing gloves using AG, AW, BF, TAS, or TAW (N=4 for each product). Additionally, the backs of wrestlers' necks were cleaned with an AW. Necks and right hands were swabbed immediately after cleaning to establish killing of bacteria at onset of practice. Wrestlers then participated in a regular 1.5-hour practice where they interacted with each other regardless of treatment type. At the end of practice, both hands and backs of necks were again swabbed and processed.

2.2. Controlled Study - Classroom

An additional study was conducted outside of wrestling practice to simulate the effect of handling every-day objects between bouts, a common occurrence during competitive meets. Twenty students swabbed their hands as described for the wrestling practice study. They then washed their hands with AG, TS, SL, ETG or BF (N=4 per product). The washed hands were swabbed to test for initial cleanliness. For 30 minutes, students passed around various items which can be commonly found in a public venue. At the end of 30 minutes, hands were again swabbed. All swabs were processed as described in section 2.4.

2.3. Competition Study – Ohio Northern University 2016 Wrestling Invitational

To determine if controlled studies were representative of effectiveness of hand cleaning in a large wrestling venue, use of hand cleansers by 193 wrestlers from 11 schools participating in the 2016 Wrestling invitational at ONU was explored. Only data from verified totally compliant wrestlers was used. All mats were cleaned with 10% bleach prior to onset of the meet and were not recleaned during the meet. Wrestlers were divided by weight classes into groups which used AG (N=34), CSF (N=40), BF (N=32), AEC (N=37). All wrestlers cleaned their hands and the backs of their necks with the product being tested according to manufacturers' guidelines for use (see Table 1). Hands were swabbed after cleaning to establish an "in" baseline and again once the wrestler completed competition in a double elimination tournament to determine the "out" load.

2.4. Swab Extraction Protocol

Bacteria were extracted from the swabs by placing swabs in tubes containing 2 ml of Mueller-Hinton broth and agitated in an Eppendorf ThermoMixer C thermoagitator at 1000 rpm for 5 minutes at 37°C. A 10 µl sample of each extract was inoculated onto trypticase soy agar with 5% sheep red blood cells and incubated for 24 hours at 37°C in 5% CO_2. This technique consistently yields a count representing 1% of bacteria swabbed. [5] Colonies per plate were counted and visual identification of predominant bacterial types present was made. *Staphylococcus aureus* was confirmed via coagulase testing.

2.5. Mat Microbiome Study – Wrestling Practice

Because not all bacteria show up in culture methods commonly used to study the wrestling environment, and because some non-culturable bacterial species are pathogenic, DNA analysis was conducted to evaluate the composition of microbial communities including both culturable and non-culturable species on wrestling mats.

Prior to wrestling practice, four mats were washed with Dawn® dish soap and water to remove any residual cleaners. Once dry, they were cleaned as follows 1: thyme-based cleaner (Benefect® Decon 30), 2: a quaternary ammonium product with residual killing activity (KenClean™), 3: 10% bleach solution, and 4: 10% bleach solution + hand sanitizing (wrestlers cleansed with AG at onset of each bout on this mat). Throughout practice, wrestlers rotated from mat to mat in 15 minute increments. Adjacent 3”x6” sections of mats were swabbed at 0 minutes (immediately before practice began), and 15, 30, 45 and 60 minutes. Swabbing of mats was conducted with synthetic swabs (MW113; Advantage Bundling) wet with 95% ethanol (four samples per mat at each time period; N=80). Swabs were preserved in 95% ethanol and kept on ice until being transferred to a -80°C freezer for storage until DNA extraction.

DNA was extracted using a bead beating (2x40 seconds) and phenol chloroform extraction method. [7] Purified DNA samples were sequenced at the University of Michigan Center for Microbial Analysis via Illumina MiSeq™ DNA sequencing (V4 target) of the 16S rRNA bacterial gene region. Samples were amplified using the dual-indexing sequencing strategy. [8] De-multiplexed paired-end sequence reads were assembled and processed using MOTHUR version 39 (5). [9] Sequences were filtered, aligned, and trimmed for chimeras. Filtered sequences were clustered by bacterial OTUs (operational taxonomic units), which are genetic sequences which have been reliably identified to a similarity threshold of 97% to the family level. Sequences were classified based upon the Green Genes database; and sequences that classified as Eukaryota, Archaea, mitochondria, and chloroplasts were removed from the dataset. Samples were rarefied to standardize sampling effort. Relative abundance data was arcsin square root transformed to account for zero inflation. [10] Sequences classified as bacteria were analyzed using permutation based analysis of variance (PERMANOVA) [11] and non-metric multidimensional scaling ordination (NMDS) [10] to assess main and interactive effects of the four cleaning protocols and time. Indicator species analysis [10] was conducted to assess drivers of bacterial community differences across treatments.

3. Results and Discussion

3.1. Results of Wrestling Practice Controlled Studies

In assessing application method, 10,368 colonies were counted of which 94.7% were *S. epidermidis*, 3.4% *S. pneumoniae*, 1.5% *S. aureus*, 0.06% *B. subtilis,* and 0.4% were grouped together as "other". Incoming wrestlers were variably clean with an initial count of 9.1 + 19.2 CFUs. All cleaners were effective in reducing load on cleaned right hand to 1.5 + 4.8 CFUs. However, the TAS only reduced the load by 50%. During practice, load rose over time, with great variability, to a mean of 48.3 + 21.5 CFUs. This count was compared to unwashed left hands (66.7 + 52.9 CFUs) and effectiveness was determined as a percent of the unwashed hands, indicating that hand cleaning does reduce bacterial load (see Figure 1).

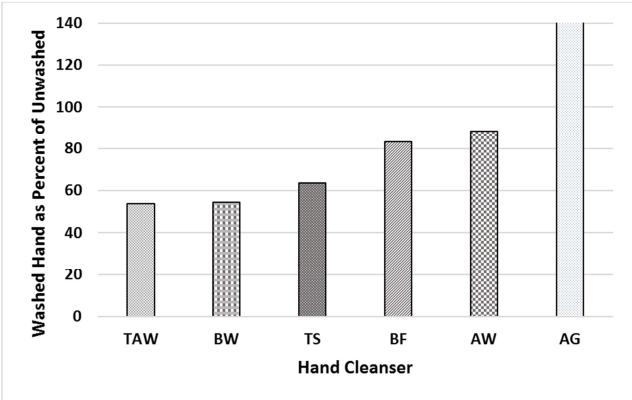

Figure 1. *Bacterial load on washed hand expressed as a percent of load on unwashed hand. Due to varied use of cleansers by participants, high SD resulted in no significant difference between treatments* $F_{(5,26)} = 0.8771$, $p=0.5100$.

Comparison of application method was made between residually active benzalkonium chloride products and non-residual alcohol based products. The BF (foam) cleaned hands had 41.2% fewer CFUs and BW (wipes) 38.9% fewer CFUs than the corresponding alcohol based cleaners indicating that cleaning with a product with residual activity may effectively reduce bacterial load and thus infection risk in the wrestling environment. Hands washed with wipes were cleaner those washed with gel/foam cleansers which is in keeping with the findings of Anderson. [12] Presumably, this was due to added value of rubbing with wipes (see Figure 1). This trend of effectiveness of residual cleansers, while not significant ($F_{(4,11)}$=0.7099, p=0.6019), was also seen in the

wrestling practice study comparing two cleaners with presumptive residual activity (TAS, BF) to two with no residual activity (CG, AG) (see Figure 2).

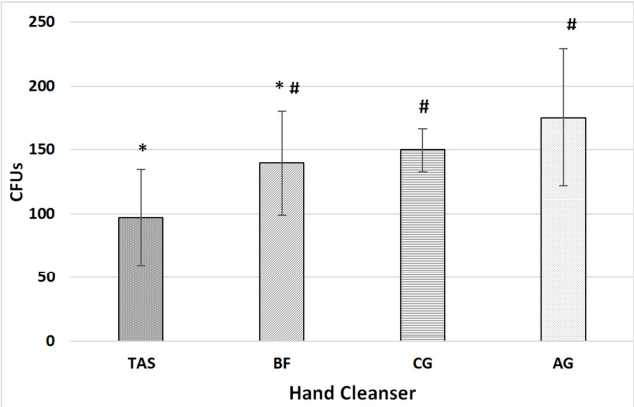

Figure 2. *Hand cleansers with residual action (TAS, BF) are more effective in reducing build-up of bacterial load than non-residual cleansers (CG, AG). Error bars indicate standard deviation.* $F_{(3,18)} = 3.1980$, *p=0.0483; post hoc T-tests TAS:BF p= 0.0663; BF:CG p=0.295; CG:AG p=0.1534.*

3.2. Results of Classroom Study

A total of 2,488 colonies were counted, consisting of 72.79% *S. epidermidis*, 26.41% *S. pneumoniae*, 0.04% *S. aureus*, 0.08% *B. subtilis* and 0.68% grouped together as "other". Students had a mean initial load of 50.45 + 51.9 CFUs per person. Load was reduced by 79% by washing of hands to 10.65 + 13.97 CFUs per person, with the TAS cleaner being the least effective (bacteria reduced to 81.9% of initial) and ETG and BF having greatest effectiveness (at 6.3% and 9.4% of initial, respectively) (see Figure 3). Further, after 30 minutes of handling miscellaneous items, CFUs rose from 10.65 + 13.97 in the clean state to 63.3 + 95.87 with greatest bacterial counts on hands washed with cleaners with no residual claims (AG, SL and TS) and least increase in bacterial load in subjects using cleaners claiming residual action (BF and ETG).

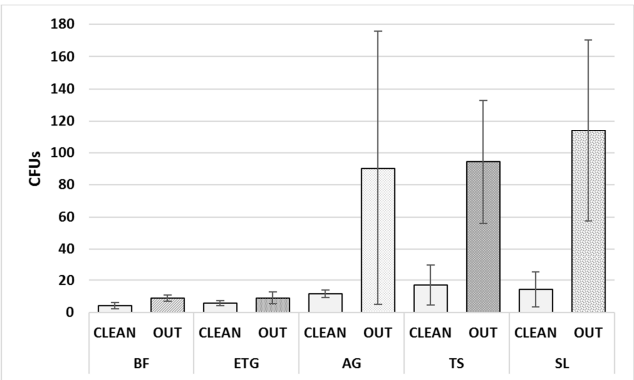

Figure 3. *Comparison of initial (clean) vs final (out) bacterial load on students passing common items to simulate between bout contamination shows huge deviations but clear trends toward residual cleaners (BF, ETG) having greater effectiveness than non-residuals (AG, SL). Lack of significance is attributed to huge deviations* $F_{(4,15)} = 1.0617$, *p= 0.4092.*

3.3. Results of Invitational

After washing, but prior to the start of the competition, wrestlers had a large variance in bacterial counts. It is unclear whether this is due to effectiveness of the products or due to conscientiousness of the wrestlers in using the product; likely the latter. Average colonies on grapplers was 2.45 + 8.9 for wrestlers using AECS (N=43); 9.47 + 19.53 for wrestlers using AG (N=40); 10.45 + 21 for wrestlers using CSF (N=48) and 18.67 + 33.8 for those using BF (N=37) ($F_{(3,164)}$=3.4615, p=0.0177). A total of 23,521 CFUs were counted at the end of the meet. Of the colonies counted, 13 species were positively identified and another 8 were classified to genus level. 82.5% were skin bacteria, predominantly *S. epidermidis*, 7.5% were respiratory bacteria, predominantly *S. pneumoniae*, 8.3% were soil bacteria, predominantly *Bacillus. spp.* and 1.6% were gut bacteria such as *E. coli*. Given the predominance of *S. epidermidis*, antibiosis by cleansers was assessed for this bacterium alone. Used as described, according to manufactures specifications (Table 1), all cleaning methods reduced bacterial load as compared to the 2014 invitational when no handwashing was conducted (see Figure 4). [5] Due to high standard deviations, no significant difference was observed between cleaning methods ($F_{(3,153)}$ = 2.376, p=0.072). These results are in agreement with meta-analysis of hand hygiene on infectious disease risk in the community setting: any form of hand cleansing is a good preventative measure against the spread of infection. [13]

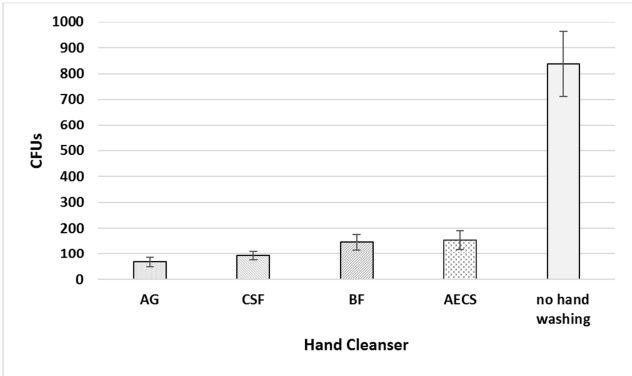

Figure 4. *Comparison of hand cleanser types used in 2016 invitational to no handwashing during 2014 invitational, showing that any form of hand washing is significantly better than no hand washing* ($F_{(4,154)}$ *=37.9748, p=4.4 x10*$^{-22}$*). But there is no significant difference between hand washing methods used in this study* ($F_{(3,139)}$ *=2.1928, p=0.0916).*

3.4. Results of Microbiome Study

Cleaning protocol had a significant effect on bacterial community structure (PERMANOVA analysis $F_{(3,72)}$= 1.58, p= 0.01). There was also a significant interaction between cleaning product and time ($F_{(3,72)}$= 1.99, p= 0.001) as is demonstrated by NMDS ordination (see Figure 5).

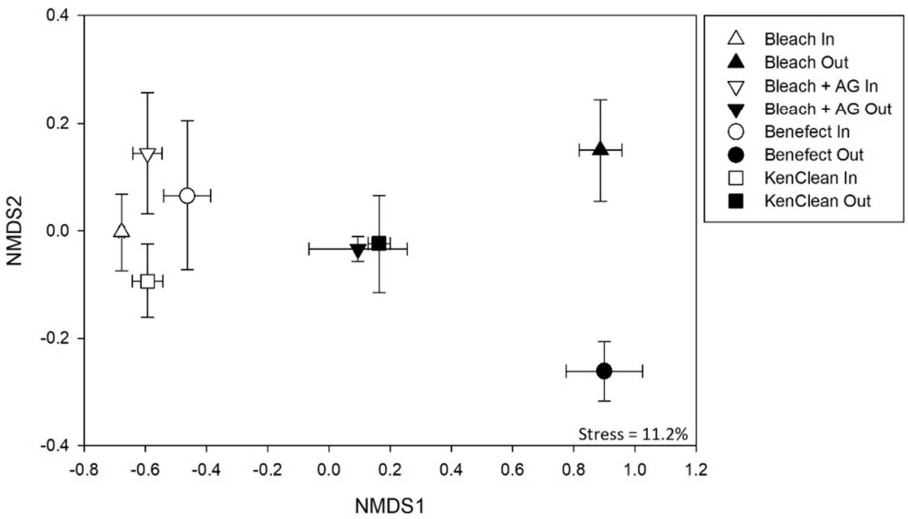

Figure 5. *NMDS ordination of bacterial community structure across wrestling mats cleaned with: 1) 10% bleach solution, 2) 10% bleach solution + hand sanitizing with AG, 3) Benefect® Decon 30, and 4) KenClean™. Points represent mean community composition with standard error, the closer two points are, the more similar their bacterial community. Plot indicates bacterial communities were similar at onset of wrestling practice (white) and bacterial communities on mats changed over time as wrestlers grappled on the mats (black = end of wrestling practice). Cleaning hands with alcohol gel when using bleach cleaned mats produced a community comparable to cleaning with the residual mat disinfectant.*

After 60 minutes, communities of bacteria on the mats cleaned with the residual quaternary ammonium compound KenClean™ and non-residual 10% bleach accompanied by hand sanitizing with AG did not differ. OTU indicators (Table 2) are valued on a scale of 0-1 such that the higher the number, the more influential the OTU is in differentiating between treatments.

Table 2. *Indicator species OTUs of bacterial organisms across wrestling mat cleaning treatments. Indicator Value (IndVal; 0-1) is provided for each cleaning treatment. P value represents the probability of obtaining the observed indicator value.*

Cleaning product	OTU	Classification (>97%)	IndVal	P value
Bleach	33	*Prevotella intermedia*	1	0.003
	38	*Prevotella sp.*	1	0.004
	56	*Sphingobacterium mizutaii*	0.87	0.011
	61	*Dialister sp.*	0.8664	0.005
	57	*Peptostreptococcus sp*	0.8217	0.002
	41	*Selenomonas sp.*	0.7956	0.008
	40	*Streptococcus sp.*	0.7571	0.01
	148	*Selenomonas sp.*	0.75	0.025
	281	*Actinomyces sp.*	0.75	0.029
	19	Staphylococcaceae	0.6947	0.004
	20	*Brevundimonas diminuta*	0.6713	0.008
	15	*Facklamia sp.*	0.6684	0.003
	18	*Aeromonas sp.*	0.6602	0.002
	28	*Fusobacterium sp.*	0.6601	0.032
	37	Staphylococcaceae	0.6279	0.027
	176	*Anaerococcus sp.*	0.5796	0.01
	93	*Psychrobacter saguinis*	0.5661	0.045
Bleach + Hand Gel	3	*Pseudomonas sp.*	0.3801	0.036
KenClean	12	*Escherichia coli*	0.4341	0.017
	16	*Corynebacterium sp.*	0.3872	0.013
	129	*Roseburia faecis*	0.75	0.031
	136	Ruminococcaceae	0.75	0.027
Benefect	11	*Acinetobacter sp.*	0.693	0.003
	68	*Collinsella aerofaciens*	0.6272	0.027
	13	*Prevotella copri*	0.6157	0.019
	71	*Kocuria sp.*	0.5574	0.048
	14	*Enhydrobacter sp.*	0.4948	0.016
	2	*Micrococcus luteus*	0.2952	0.03

Difference between cleaning protocols was found to be dependent on four non-pathogenic OTUs [0001 *Pseudomonas fragi* (75% surety of resolution); 0002 *Micrococcus luteus* (99%); 0003 *Pseudomonas veronii* (72%); 0008 *Streptococcus infantis* (85%)] which comprised the 80% most abundant organisms. It should be noted that

species which are non-pathogenic were classified as such, but those which are opportunistic pathogens were classified as pathogenic to err on the side of caution. The addition of the hand cleaning regime to bleach cleaned mats (Bleach plus AG) results in bacterial communities similar to those found on mats cleaned with the residual cleanser, KenClean™ and have strikingly fewer pathogenic indicator OTUs than the bleach treatment alone (see Figure 6).

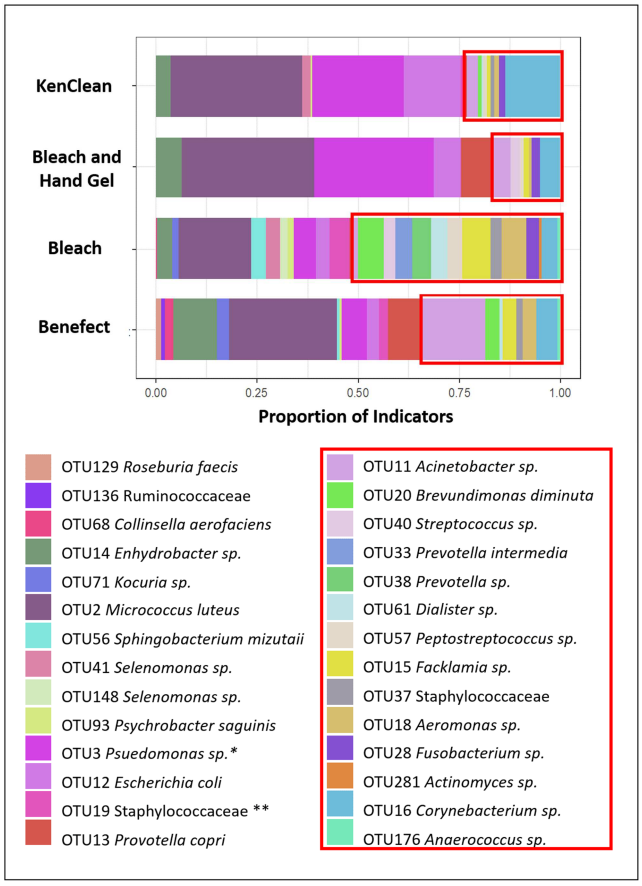

Figure 6. *Indicator species of treatment differences. Potentially pathogenic organisms boxed in red. Classifications in table >97% confidence (Green Genes database) except *non-pathogenic Pseudomonas veronii; 72%. resolution confidence, ** non-pathogenic Jeotgalicoccus sychrophilus, 84% resolution confidence.*

4. Conclusions

The results of this investigation reinforce previous findings that support use of residual cleaners to disinfect mats. Furthermore, the addition of hand cleansing (using any of the forms of hand sanitizer tested) to the mat cleaning regimen (regardless of disinfectant type) can effectively reduce bacterial populations on wrestlers and alter bacterial community structure on the mats. Thus hand cleansing should reduce transmission of bacteria from mats to wrestlers, and consequently infection risk.

Acknowledgements

The authors would like to thank Assistant ONU Wrestling Coach Kyle Kwiat and Research Assistant Suzy Young for logistics support, April Cockburn of the University of Michigan Center for Microbial Analyses for DNA sequencing, and Mike Moyer and the NWCA for funding this research.

References

[1] Selling, B., and S. Kibrick. (1964) An Outbreak of Herpes Simplex among Wrestlers (Herpes Gladiatorum). New England Journal of Medicine, Massachusetts Medical Society.

[2] Agel, J., J. Ransone, R. Dick, R. Oppliger, and S. W. Marshall. (2007) Descriptive Epidemiology of Collegiate Men's Wrestling Injuries: National Collegiate Athletic Association Injury Surveillance System, 1988–1989 Through 2003–2004. Journal of Athletic Training. Apr-Jun; 42 (2), pp. 303–310. PMCID: PMC1941299.

[3] Adams, B. B. (2002) *Tinea corporis gladiatorum*. Journal of the American Academy of Dermatology 47 (2), pp. 286–290.

[4] Stanforth, B., A. Krause, C. Starkey, and T. J. Ryan. (2010) Prevalence of Community- Associated Methicillin-Resistant *Staphylococcus aureus* in High School Wrestling Environments. Journal of Environmental Health. 72 (6), pp. 12-16.

[5] Young, L. M., V A. Motz, E. R. Markey, S. C. Young, and R. E. Beaschler. Recommendations for Best Disinfectant Practices to Reduce the Spread of Infection via Wrestling Mats. Journal of Athletic Training. 52 (2), pp. 82–88.

[6] Habibipour, R., L. Moradi-Haghgou, and S. Bayat. (2012) Survey on Dermatophytosis in Wrestlers and Its Relationship with Wrestling Mats in Hamedan. Zahedan Journal of Research Medical Science. 14 (8), pp. 38–42.

[7] Burke, D. J., K. A. Smemo, J. C. Lopez-Gutierrez, and J. L. DeForest. (2012) Soil Fungi Influence the Distribution of 196 Microbial Functional Groups that Mediate Forest Greenhouse Gas Emissions. Soil Biology and Biochemistry. 197 (53), pp. 112-119.

[8] Kozich, J. J., S. L. Westcott, N. T. Baxter, S. K. Highlander, and P. D. Schloss. (2013) Development of a Dual-Index 211 Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina 212 Sequencing Platform. Applied and Environmental Microbiology. 79, pp. 5112-5120.

[9] Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, J. W. Sahl, B. Stres, G. G. Thallinger, D. J. Van Horn, and C. F. Weber. (2009) Introducing MOTHUR: Open-source, Platform-independent, Community-supported Software for Describing and Comparing Microbial Communities. Applications in Environmental Microbiology. 75 (23), pp. 7537-41. doi: 10.1128/AEM.01541-09.

[10] McCune, B., J. Grace, and D. Urban. (2002) Analysis of Ecological Communities. MjM Software Design, Gleneden Beach, Oregon.

[11] Oksanen J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, H. Steven, E. Szoecs, and H. Wagner. Vegan: Community Ecology Package. R Package Version. 224 (2), pp. 4-2.

[12] Anderson, B. J. (2012) Effectiveness of Body Wipes as an Adjunct to Reducing Skin Infections in High School Wrestlers. Clinical Journal of Sport Medicine. 22 (5), pp. 424–429.

[13] Aiello, A. E., R. M. Coulborn,, V. Perez, and E. L. Larson. (2007) Effect of Hand Hygiene on Infectious Disease Risk in the Community Setting: A Meta-Analysis. American Journal of Public Health (AJPH) AJPH. 2007. 124610.