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Distribution of *Staphylococcus* species in dairy cows, workers and shared farm environments

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One sentence summary: The study took a "One Health" approach by examining staphylococci in humans, animals, as well as, the environment, in order to better understand the interactions of microbial communities and the possible impact of such interactions.

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ABSTRACT

Dairy farming involves frequent contact among animals, workers and farm environments. To explore the *Staphylococcus* spp. diversity that occurs on dairy farms, a pilot study sampled dairy workers, cows and the farm environments from five farms, two organic and three conventional farms, in Washington State. Samples were taken from the nares and hands of consenting workers (n = 24), udders and nares of selected cows (n = 25) and representative environmental surfaces (n = 96) from each farm. To increase diversity of the *Staphylococcus* spp. characterized, five distinct colonies were selected from each sample for identification with 16S analysis. A total of 198 staphylococci were characterized representing 19 different *Staphylococcus* spp. The diversity of species ranged from 9–15 *Staphylococcus* spp./farm with no difference between conventional and organic farms. *S. haemolyticus* [n = 60 isolates] was the most common species and was isolated from all farms and from cows, humans and environmental samples. Whole genome sequencing of selected *S. haemolyticus* found no genetically related isolates among human, animal and environmental samples within the same farm. *S. epidermidis*, *S. saprophyticus*, *S. sciuri* and *S. xylosus* were also found in ≥1 farms from human, animal and environmental samples.

Keywords: *Staphylococcus* spp.; *S. haemolyticus*; dairy; environment

INTRODUCTION

Dairy farming involves direct contact between workers and cows during milking and moving animals, as well as, exposures to manure, dust and liquid splashes all of which allow for the potential bacterial transmission between humans, cows and the farm environment. The level of human-cow interaction leading to bacterial transfer in dairy farm operations is not clear. Studies have illustrated an inconsistency in infection control practices including variable use of gloves and other personal protective equipment [PPE] when handling animals and hand washing after animal contact (Fenton *et al.* 2010b). The lack of PPE use may explain why there have been studies of dairy workers associating a variety of skin disorders and gastrointestinal infections related to work in milking barns and contact with dairy cow feces (Gilpin *et al.* 2008; Fenton *et al.* 2010a; Awadallah *et al.* 2016). Case reports have documented the development of skin

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Table 1. Farm Characteristics and Management Practices.

Farm	Organic vs. conventional	Herd Size	Total # workers	# Workers sampled	Pasture access	Manure management	Non-therapeutic use antibiotics
1	Conventional	7000	52	7	No	Hauled and spread daily, composted, lagoon storage (time of yr dependent)	No
2	Conventional	875	9	3	Summer only	Composted, lagoon storage	No
3	Organic	409	10	6	Yes	Hauled and spread daily, composted, lagoon storage (time of yr dependent)	No
4	Organic	185	5	1	Yes	Lagoon storage	No
5	Conventional	2200	22	7	Summer only	Composted, lagoon storage	Yes

infections with *Staphylococcus* spp. among dairy workers and family contacts (Tiwari and Tiwari 2007).

Staphylococcus aureus has been associated with mastitis in cows (Keefe 2012; Boss *et al.* 2016; Capra *et al.* 2017), but now other *Staphylococcus* spp. are associated with mastitis as well (Keefe 2012; Kateete *et al.* 2013; De Visscher *et al.* 2014; Srednik *et al.* 2017). As other species of *Staphylococcus* become more commonly associated with disease it is of interest to learn more about the distribution of these coagulase negative *Staphylococcus* spp. [CoNS] in dairy farms (Levison *et al.* 2016; Nyman, Fasth and Waller 2018).

In the current pilot study, we sampled five dairy farms [3 conventional and 2 organic] to assess the degree of diversity and similarities among *Staphylococcus* spp. isolated from cow noses and teats, human noses and hands and a variety of dairy farm environments. Generalized growth for *Staphylococcus* spp. was done rather than specific enrichment for *S. aureus*. We determined whether there were differences in *Staphylococcus* spp. present and/or number found in humans, cows and the farm environment. We also determined whether for *S. haemolyticus*, the most commonly isolated species, there was phylogenetic relatedness between isolates from different types of samples (human, animal and environment) on the same farm.

MATERIALS AND METHODS

Recruitment of farms and workers

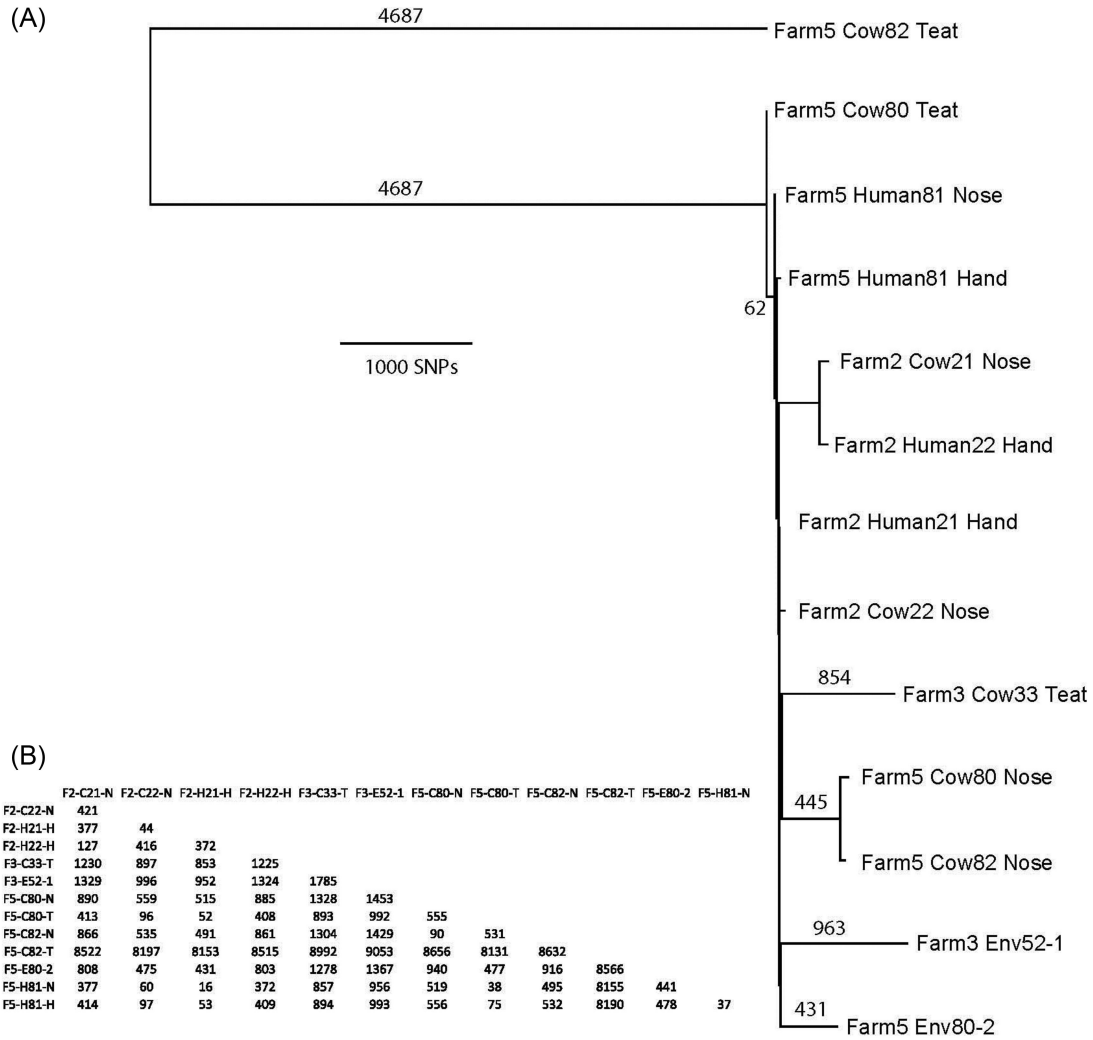
We recruited five dairy farms in Washington State through the assistance of a number of professional dairy-related contacts in the area. These farms represented a convenience sample with herd sizes ranging from 175 to 7000 head of cattle. Three out of the five farms were considered conventional production systems with the remaining two following under the organic classification (Table 1). After gaining owner permission to visit the farms, samples were collected from cows [one nose and one teat sample from each cow and five cows from each farm] and 18–20 environment samples per farm. All cows were sampled in the milking barn during milking operations. Thus none were known to be sick or on antibiotic treatment at the time of sampling. Sampling took place between May 2014– Feb 2015. Almost all workers wore gloves during milking. Farm workers were invited to volunteer to participate in the survey and sampling. Study protocols were reviewed and approved by the University of Washington human subjects review board (#46 454) as well as the Institutional Animal Care and Use Committee (IACUC) committee (#4335–01).

Microbial sampling of workers, cows and environments

The farms included in the study volunteered to be in the study and included the first five that agreed to be sampled. For feasibility in this pilot study, five cows from each farm were selected for sampling, as well as, all workers who consented and could be sampled during a field visit. We planned environmental sampling based on taking at least two samples for each of a number of high contact environments, such as enclosure railings, milking areas and feeding areas. The number of samples taken also factored in how many samples could be processed in a single time period. Nasal and hand swabs were collected from human participants by trained study staff using SANICULT™ Transport Swabs (Roberts *et al.* 2011). Nasal and teat swabs were collected from cows currently in lactation by a veterinarian or trained study staff member using 3M™ Sponge-Stick with neutralizing buffer (3M Science Applied to Life, USA). From each farm, 18–20 environmental samples were taken; including surface swabs (milking equipment, washing machine and sinks in the milking barn, towels to wipe the teats, etc.) and environmental media (manure runoff, bedding, feed, etc.) from each farm using previously established methods (Soge *et al.* 2016). The same surfaces and environmental media were sampled at each farm. All swab samples were transferred to the laboratory where they were processed within 24 h of collection as previously described (Roberts *et al.* 2011; Soge *et al.* 2016).

Microbial analysis

Samples were enriched in Bacto® m Staphylococcus Broth (Difco Laboratories, Division BD Sparks, MD), supplemented with 75 mg/L polymyxin B and 0.01% potassium tellurite (Sigma-Aldrich, St. Louis, MO) and incubated at 36.5°C with 5% CO_2 (Soge et al. 2009, 2016; Roberts *et al.* 2011; Michael, No and Roberts 2016). At 48 h, all tubes with growth and black precipitate were streaked for isolation on Staphylococcus medium 110 (Difco Laboratories), as previously described (Soge et al. 2009, 2016; Roberts *et al.* 2011; Michael, No and Roberts 2016). One to five unique colonies were selected from each plate and streaked on Brucella agar supplemented with 5% sterile sheep blood (Difco Laboratories) and incubated at 36.5°C in 5% CO_2 for 24 h to determine colony morphology [rough vs smooth], colony color [white to gray or yellow] and hemolysin production (Michael, No and Roberts 2016). Some samples had <5 *Staphylococcus* spp. on the plate and thus had <5 colonies selected for characterization. When there was multiple isolates selected and identified from the same sample, especially with *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus* and *S. xylosus* only one isolate from any one species was selected for inclusion in the study to prevent duplication of the same strain.



(B)

	F2-C21-N	F2-C22-N	F2-H21-H	F2-H22-H	F3-C33-T	F3-E52-1	F5-C80-N	F5-C80-T	F5-C82-N	F5-C82-T	F5-E80-2	F5-H81-N
F2-C22-N	421											
F2-H21-H	377	44										
F2-H22-H	127	416	372									
F3-C33-T	1230	897	853	1225								
F3-E52-1	1329	996	952	1324	1785							
F5-C80-N	890	559	515	885	1328	1453						
F5-C80-T	413	96	52	408	893	992	555					
F5-C82-N	866	535	491	861	1304	1429	90	531				
F5-C82-T	8522	8197	8153	8515	8992	9053	8656	8131	8632			
F5-E80-2	808	475	431	803	1278	1367	940	477	916	8566		
F5-H81-N	377	60	16	372	857	956	519	38	495	8155	441	
F5-H81-H	414	97	53	409	894	993	556	75	532	8190	478	37

Figure 1. Phylogenetic analysis reveals distant relationship among isolates. (A) Core genome SNP phylogeny of 13 *Staphylococcus haemolyticus* isolates sequenced in this study. Branch lengths are labeled with the number of SNP differences. (B) Pairwise SNP difference map of isolates sequenced in this study. The SNP differences are consistent with a lack of recent transmission between cow, workers and the environment.

This happened most often in the environmental samples. All the coagulase-negative isolates either had no hemolysin or α-hemolysin, while the six β-hemolytic isolates, in the study, were identified as *S. aureus* using 16S sequencing and the Staphaurex test as previously described (Remel, Lenexa, KS; Roberts *et al.* 2011).

Initially, isolates were tested using the API STAPH-IDENT (BioMerieux, USA) because of relatively good identification of coagulase-negative *Staphylococcus* spp. (Ameida and Jorgensen 1983). However, >50% of the initial isolates could not be adequately identified. Therefore, we chose to test all isolates using PCR assays for 16S sequencing using Proteinase K (pK) treatment cells as previously described using the Standard Sanger method (Soge et al. 2009, 2016; Roberts *et al.* 2011). The PCR amplicons were sequenced in both directions by Eurofins Genomics (Louisville, KY) and compared to the 16S database to characterize *Staphylococcus* spp. isolates as previously described (Soge *et al.* 2009). Only isolates that showed high homology in the 16S PCR assay, 99–100%, were included in the study. In addition, if any isolate show sequence homology to ≥1 species that isolate was not included in the final study to increase the accuracy of the coagulase-negative *Staphylococcus* spp.(CoNS) identification. All of the isolates that gave good identification using the API system had the same species identification using the 16S PCR system. With this system, 198 isolates were speciated and used for further testing.

Whole genome sequencing

DNA was prepared from the 13 *S. haemolyticus* for Whole Genome Sequencing (WGS) using UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories, Inc. now owned by QIAGEN Inc. Germantown, MD). The *S. haemolyticus* came from three different farms, all of which had *S. haemolyticus* isolated from cows, humans and the environment (Table 3). One pair of isolates from the same person's nose and hand were selected to determine if they carried a single clone. Then randomly selected *S. haemolyticus* isolates from cow nose and teat samples, human hand and nose and two environmental sites were included for comparison (Figure 1). Creation of whole genome libraries was performed as previously described (Greninger, Langelier and Cunningham, 2015, 2016). One ng of bacterial genomic DNA [gDNA] was used as input for dual-indexed Nextera XT library preparation with 14 cycles of amplification and sequenced on a 1 × 185bp run

Table 2. *Staphylococcus* spp. Identified, by Sampling Type.

Staphylococcus spp. identification	Cow nose (n = 25)	Cow teat (n = 25)	Human hand (n = 24)	Human nose (n = 24)	Environment surface (n = 48)	Environment media (n = 48)	Total
S. arlettae	–	1	–	–	1	–	2
S. aureus	1	–	1	4	–	–	6
S. chromogenes	2	4	–	1	–	1	8
S. cohnii	1	1	–	1	–	–	3
S. devriesei	–	1	1	–	–	1	3
S. epidermidis	3	2	2	8	–	4	19
S. equorum	1	–	–	1	2	3	7
S. fleurettii	1	–	1	–	–	–	2
S. haemolyticus	10	7	9	5	15	14	60
S. hominis	–	–	1	1	1	–	3
S. lentus	–	–	–	–	1	2	3
S. nepalensis	–	–	–	–	2	–	1
S. pasteuri	–	–	2	–	–	–	2
S. saprophyticus	2	–	4	7	1	8	21
S. sciuri	6	3	–	1	–	5	15
S. succinus	1	1	–	–	1	12	15
S. vitulinus	–	–	1	–	–	–	1
S. warneri	–	–	–	–	–	1	1
S. xylosus	5	5	4	2	8	–	24
TOTALS	33	25	26	31	32	51	198

on an Illumina MiSeq to achieve approximately 1 million reads per isolate. Reads were trimmed using trimmomatic, de novo assembled using SPAdes v3.11 and annotated using prokka v1.11 (Bankevich *et al.* 2012; Bolger, Lohse and Usadel 2014; Seemann 2014). Core genome hqSNP phylogenies were created as described previously using bwa-mem, samtools and vcftools based on mapping to the *S. haemolyticus* strain JCSC1435 reference genome [NC_0 07168] using minDP 10, minQ 200 and minGQ 10 parameters in vcftools (Li and Durbin 2010; Kozyreva *et al.* 2016; Goncalves da Silva *et al.* 2017). In this manner, WGS was performed on 13 *S. haemolyticus* isolates. A median of 1,036,488 reads [range 637 812–2297 414 reads] were sequenced per isolate, yielding a median depth of 69X [range 42–154X]. The Whole Genome Shotgun project has been deposited at DDBI/ENA/GenBank under the access PRJNA450870. Accession # are SAMN08950978 [isolate Farm 5 Cow82 Teat] to SAMN0895090 [isolate Farm 5 Env80–2].

Data analysis

We performed descriptive analysis of isolate frequencies in animal, human and environmental samples at the farm level. In manuscript preparation, we followed the COHERE checklist for one health epidemiological reporting of evidence for One Health studies (Davis et al. 2017).

RESULTS

Farm characteristics

The characteristics of the five farms in the study are in Table 1. Year round pasture access took place only in the two organic farms, while seasonal access to pasture took place on two of the three conventional farms. All farms used manure lagoons as part of waste management.

Isolation and characterization of *Staphylococcus* spp.

A total of 24 workers agreed to be sampled, with a range of 1–7 workers/farm (Table 1). A total of 194 samples were collected during the study and included: 50 cow samples (25 nasal, 25 teat), 48 human samples (24 nasal, 24 hands) and 96 environmental samples. From these 198 different staphylococci were isolated including; 33 cow nasal and 24 cow teat isolates, 26 human hand and 31 human nasal isolates and 83 environmental isolates. These isolates were classified into 19 different *Staphylococcus* spp. (Table 2). On the organic dairies, the number of different species of *Staphylococcus* ranged from 9–11 different species while conventional dairies had between 10–15 different *Staphylococcus* spp. We found 4–7 different *Staphylococcus* spp. among the cow samples, 2–9 different *Staphylococcus* spp. among the worker samples, and 6–8 different *Staphylococcus* spp. in the environmental samples (Table 3). For the environmental samples, 32 positive *Staphylococcus* spp. isolates came from swab surface samples and 51 isolates from environmental media (Table 2).

S. haemolyticus was the most commonly isolated *Staphylococcus* spp. overall (n = 60), followed by *S. xylosus* (n = 24), *S. saprophyticus* (n = 21), *S. sciuri* (n = 15) and *S. succinus* (n = 15) (Table 2). The *S. haemolyticus* included: 17 isolates from cows, 14 isolates from human and 29 isolates from the environment (Table 3). In Farms #2, #3 and #5, *S. haemolyticus* was isolated in cow, human and environmental samples. While in Farm #1, *S. haemolyticus* was isolated from human and environmental samples and Farm #4 isolated from cow and environmental samples. *S. epidermidis* isolates were cultured from Farm #5 from all three sectors (Table 3). From organic Farm #3, *S. haemolyticus*, *S. saprophyticus* and *S. xylosus* were isolated in all three sectors [cow, human and environment]. The organic Farm #4 had *S. saprophyticus* and *S. xylosus* isolated in all three sectors, while conventional farms had ≥1 of the following in all three sectors; *S. haemolyticus*, *S. saprophyticus* or *S. epidemidis* (Table 3).

Table 3. *Staphylococcus* spp., Across Sectors, by Farm.

	Cow	Human	Environment
Farm 1			
	S. chromogenes		*S. chromogenes*
	S. cohnii		
		S. epidermidis	
Cow (5)		*S. haemolyticus*	*S. haemolyticus*
Human (7)		*S. hominis*	
Environment (17)			*S. lentus*
			S. nepalensis
	S. saprophyticus	**S. saprophyticus**	**S. saprophyticus**
	S. sciuri		
			S. xylosus
Farm 2	*S. aureus*	*S. aureus*	
	S. chromogenes	*S. chromogenes*	
	S. cohnii		*S. cohnii*
		S. epidermidis	*S. epidermidis*
Cow (5)			*S. equorum*
Human (3)	**S. haemolyticus**	**S. haemolyticus**	**S. haemolyticus**
Environment (19)		*S. pasteuri*	
			S. saprophyticus
			S. sciuri
	S. xylosus	*S. xylosus*	
Farm 3		*S. aureus*	
	S. devriesei	*S. devriesei*	
		S. epidermidis	
			S. equorum
Cow (5)	*S. fleurettii*		
Human (6)	**S. haemolyticus**	**S. haemolyticus**	**S. haemolyticus**
Environment (20)			*S. hominis*
	S. saprophyticus		*S. saprophyticus*
	S. sciuri	**S. sciuri**	**S. sciuri**
	S. succinus		*S. succinus*
	S. xylosus	**S. xylosus**	**S. xylosus**
Farm 4	*S. arlettae*		
			S. devriesei
	S. epidermidis		
			S. equorum
Cow (5)	*S. haemolyticus*		*S. haemolyticus*
Human (1)	**S. saprophyticus**	**S. saprophyticus**	**S. saprophyticus**
Environment (20)	*S. sciuri*		*S. sciuri*
	S. succinus		*S. succinus*
	S. xylosus	**S. xylosus**	**S. xylosus**
Farm 5			*S. arlettae*
		S. aureus	
	S. chromogenes	**S. epidermidis**	**S. epidermidis**
Cow (5)	**S. epidermidis**	*S. equorum*	
Human (7)	*S. equorum*	*S. fleurettii*	
Environment (20)	**S. haemolyticus**	**S. haemolyticus**	**S. haemolyticus**
			S. lentus
		S. pasteuri	
		S. saprophyticus	*S. saprophyticus*
			S. sciuri
			S. succinus
		S. vitulinus	
			S. warneri
		S. xylosus	

Species in bold were found in human, cow and environmental samples at the same individual farm.

There were six *S. aureus* isolates identified, of which five were from workers and one from a cow nose. No *S. aureus* was isolated in Farms #1 and #4. In Farm #2 (conventional) one cow nose and one human *S. aureus* isolate was cultured. In Farms #3 (organic) and #5 (conventional) only human *S. aureus* isolates were identified (Table 3).

Whole genome analysis of *S. haemolyticus*

Whole genome sequencing was done on 13 selected *S. haemolyticus* isolates (Farm #5, 4 cow and 2 human; Farm #2, 2 cow and 3 humans; Farm #3, 2 environmental isolates) (Figure 1). The core genome hqSNP phylogeny revealed a median 830 SNPs [range, 33–9053 SNPs] between isolates, consistent with a lack of recent transmission with the bar representing 1000 SNP differences (Figure 1). Two isolates with 16 SNP differences were isolated from the hand and nose of human 81 at the same time. These differences suggests that these two isolates were genetically related. The phylogenetic tree showed some grouping by farm of *S. haemolyticus*, however, the overall phylogeny, in terms of SNP distances, demonstrated minimal clustering of isolates by farm.

DISCUSSION

This pilot study aimed to characterize the diversity of the staphylococcal populations and their movement within a farm setting between humans, animals and the environment. A total of 19 different *Staphylococcus* spp. from five dairy farms in Washington were identified. There was co-occurrence of *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, *S. sciuri* and *S. xylosus* across humans, cows and environments on ≥1 farms. There were no significant differences in the number of different *Staphylococcus* spp. identified in cows, environment or workers on organic vs conventional farms (Table 2). However, the number of farms was not large and it is possible that with a larger number of farms there may be a trend with specific *Staphylococcus* spp. associated with either organic or conventional farms. Finding of certain species occurring simultaneously in cows, workers and the dairy environment suggest the potential for transmission of the same bacteria between the three sectors. However, the WGS data from *S. haemolyticus* did not show a close genetic relationship between isolates taken from cows, the environment and the dairy workers on any one farm (Figure 1). The number of isolates examined in the study was low and it is possible that if larger numbers of *S. haemolyticus* were isolated and characterized some genetic relationships between isolates within a farm might be found as has occurred with *S. aureus* ST398 between pigs and pig farm workers (Reynaga *et al.* 2016). There are other strain types of *S. aureus* found among cows around the world, however only a few are common and limited studies have been performed in the USA (Price *et al.* 2012; Li *et al.* 2017).

The finding of coagulase-negative *Staphylococcus* spp. (CoNS) in cow, workers and the dairy environment is not surprising, since previous reports have indicated that in some settings CoNS are becoming the most common causes of intramammary infections in cows. For example, in one European study on three farms, both *S. haemolyticus* and *S. sciuri* were identified in all three herds, and were frequently found in milk samples (De Visscher *et al.* 2014). Another study in Uganda, found CoNS in human nasal samples from dairymen and cows with clinical mastitis though the strains in humans and cows were genetically different (Kateete *et al.* 2013) as occurred in the current study.

The abundance of *S. aureus* in the cows, humans and environmental samples were likely under estimated because we did not specifically enrich or select for *S. aureus*. This hypothesis is supported by the low [10%] of the human samples that were *S. aureus* positive. This value is at the lower end of carriage rate found in general populations (Hanis *et al.* 2017). By focusing on *Staphylococcus* spp. and isolating only the most common species that were present and able to grow with the current methods, we would not have detected low levels of other species of *Staphylococcus* spp. We did not look for the presence of the *mecA* gene or antibiotic resistance, thus it is unclear what level of antibiotic resistance the CoNS carried in the current study.

The study took a 'One Health' approach by examining staphylococci in humans, animals, as well as, the environment, in order to better understand the interactions of microbial communities and the possible impact of such interactions. This approach required assembling a study team with expertise in human, animal, environmental health and sampling methods. In future studies, it would be of value to culture and characterize both CoNS and *S. aureus*, as well as, determine antibiotic resistant phenotypes and genotypes. The increase in the number of isolates characterized in a longitudinal fashion along with increased number of organic and conventional dairy farms would help better characterize if there is CoNS movement between dairy cows, workers and their farm environment.

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REFERENCES

Ameida RJ, Jorgensen JH. Identification of coagulase-negative staphylocci with the API STAPH-IDENT system. *J Clin Microbiol* 1983;**18**:254–7.

Awadallah MA, Ahmed HA, Merwad AM *et al.* Occurrence, genotyping, shiga toxin genes and associated risk factors of *E. coli* isolated from dairy farms, handlers and milk consumers. *Vet Journ* 2016;**217**:83–8.

Bankevich A, Nurk S, Antipov D *et al.* SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;**19**:455–77.

Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinform* 2014;**30**:2114–20.

Boss R, Cosandey A, Luini M *et al.* Bovine *Staphylococcus aureus*: Subtyping, evolution, and zoonotic transfer. *J Dairy Sci* 2016;**99**:515–28.

Capra E, Cremonesi P, Pietrelli A *et al.* Genomic and transcriptomic comparison between *Staphylococcus aureus* strains associated with high and low within herd prevalence of intramammary infection. *BMC Microbiol* 2017;**17**:21.

Davis MF, Rankin SC, Schurer JM *et al.* Checklist for one health epidemiological reporting of evidence (COHERE). *One Health* 2017;**4**:14–21.

De Visscher A, Supre K, Haesebrouck F *et al.* Further evidence for the existence of environmental and host-associated species

of coagulase-negative staphylococci in dairy cattle. *Vet Microbiol* 2014;**172**:466–74.

Fenton G, Brasier K, Henning G *et al.* Occupational health behaviors and habits of women on dairy farms in Pennsylvania. *J Agromedicine* 2010;**15**:16–23.

Fenton G, Brasier K, Henning G *et al.* Occupational health characteristics of women on dairy farms in Pennsylvania. *J Agromedicine* 2010;**5**:7–15.

Gilpin BJ, Scholes P, Robeson B *et al.* The transmission of thermotolerant *Campylobacter* spp. to people living or working on dairy farms in New Zealand. *Zoon Publ Health* 2008;**55**:352–60.

Greninger AL, Chatterjee SS, Chan LC *et al.* Whole-genome sequencing of methicillin-resistant *Staphylococcus aureus* resistant to fifth-generation cephalosporins reveals potential non-*mecA* mechanisms of resistance. *PLoS One* 2016;**11**:e0149541.

Greninger AL, Langelier C, Cunningham G. Two rapidly growing mycobacterial species isolated from a brain abscess: First whole-genome sequences of *Mycobacterium immunogenum* and *Mycobacterium llatzerense*. *J Clin Microbiol* 2015;**53**:2374–7.

Goncalves da Silva A, Baines SL, Carter GP *et al.* A phylogenomic framework for assessing the global emergence and evolution of clonal complex 398 methicillin-resistant *Staphylococcus* aureus. *Microbial Gen* 2017, doi 10.1099/mgen.0.000105.

Hanis CL, Garrett KE, Essignmann HT *et al.* Hosehold aggregation of *Staphylococcus aureus* by clonal complex and methicillin resistance profiles in Starr County, Texas. *Eur J Clin Microbiol Infect Dis* 2017;**36**:1787–93.

Kateete DP, Kabugo U, Baluku H *et al.* Prevalence and antimicrobial susceptibility patterns of bacteria from milkmen and cows with clinical mastitis in and around Kampala, Uganda. *PLoS One* 2013;**8**:e63413.

Keefe G. Update on control of *Staphylococcus aureus* and *Streptococcus agalactiae* for management of mastitis. *Vet Clin N Amer: Food Anim Prac* 2012;**28**:203–16.

Kozyreva VK, Jospin G, Greninger AL *et al.* Recent outbreaks of Shigellosis in California caused by two distinct populations of *Shigella sonnei* with either increased virulence or fluoroquinolone resistance. *mSphere* 2016;**1**:e00344–16.

Levison LJ, Miller-Cushon EK, Tucker AL *et al.* Incidence rate of pathogen-specific clinical mastitis on conventional and organic Canadian dairy farms. *J Dairy Sci* 2016;**99**:1341–50.

Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinform* 2010;**26**:589–95.

Li T, Lu H., Wang X *et al.* Molecular characteristics of *Staphylococcus aureus* causing bovine mastitis between 2014 and 2015. *Front Cell Infect Microbiol* 2017;**7**:127.

Michael KE, No D, Roberts MC. Methicillin-resistant *Staphylococcus aureus* isolates from surfaces and personnel at a hospital laundry facility. *J Appl Microbiol* 2016;**121**:846–54.

Nyman AK, Fasth C, Waller KP. Intramammary infections with different non-aureus staphylococci in dairy cows. *J Dairy Sci* 2018;**101**:1403–18.

Price LB, Stegger M, Hasman H *et al.* *Staphylococcus aureus* CC398: Host adaptation and emergence of methicillin reistnace in livestock. *mBio* 2012;**3**:e00305–11.

Reynaga E, Navarro M, Vilamala A *et al.* Prevalence of colonization by methicillin-resistant Staphylococcus aureus ST398 in pigs and pig farm workers in an area of Catalonia, Spain. *BMC Infect Dis* 2016;**16**:716.

Roberts MC, Soge OO, No D *et al.* Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from fire stations in two northwest fire districts. *Am J Infect Control* 2011;**39**:382–9.

Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinform* 2014:**30**:2068–9.

Soge OO, Meschke JS, No D *et al.* Characterization of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative *Staphylococcus* spp. isolated from US West Coast public marine beaches. *J Antimicrob Chemother* 2009;**64**:1148–55.

Soge OO, No D, Michael K *et al.* Transmission of MDR MRSA between primates, their environment and personnel at a United States primate centre. *J Antimicrob Chemother* 2016;**71**:2798–803.

Srednik M, Tremblay Y, Labrie J *et al.* Biofilm formation and antimicrobial resistance genes of coagulase-negative staphylococci isolated from cows with mastitis in Argentina. *FEMS Microbiol Lett* 2017;**364**:1–8.

Tiwari JG, Tiwari HK. Staphylococcal zoonosis on dairy farms in Assam and Meghalaya. *Indian J Pub Health* 2007;**51**:97–100.