Staphylococcus, not MRSA? A Final Report of Carriage and Conversion Rates in Nursing Students

RODNEY E. ROHDE, TOM PATTERSON, BARBARA COVINGTON, BOB EDWARD VÁSQUEZ, GERALD REDWINE, EMILLIO CARRANCO

ABSTRACT

OBJECTIVE: To evaluate and characterize staphylococcal carriage, possibly including methicillin-resistant *Staphylococcus aureus* (MRSA), and conversion rates in nursing students across clinical semester rotations and to describe risk factors.

DESIGN: A prospective longitudinal cohort design with six times of measurement. Data collected August 2010 to May 2012. Institutional Review Board approval (2010F5693)

SETTING: Texas State University, San Marcos, TX

PARTICIPANTS: Eighty-seven nursing students.

INTERVENTIONS: A positive MRSA swab was considered an end point for participation. Intervention offered was bactroban (mupirocin) for nasal decolonization and an oral antibiotic, doxycycline; follow-up post treatment collection sample was done to verify decolonization prior to next clinical rotation.

MAIN OUTCOME MEASURES: Screening for *Staphylococcus aureus* and MRSA identification; confirmation and antibiotic susceptibility by Vitek 2; self-administered questionnaires delineating demographics and risk factors; panel logistic regression models by Stata version 13

RESULTS: MRSA colonization did not increase. *S. aureus* incidence was 17.7 – 26.4%. Staphylococcal species incidence other than *S. aureus* increased (9.2 – 82.3%). The following odds ratio (OR) associations were found to be statistically significant: boil or skin infections with *S. aureus* (OR = 2.94, $p < .01$), working or volunteering in a healthcare facility odds with species other than *S. aureus* (OR = 4.41, $p < .01$) and gym and sports facilities odds with *S.* other (OR 2.45, $p < .01$). The most frequently occurring species at Wave 5 was *S. hominis* (21 isolates) while the most frequently occurring species at Wave 6 was *S. epidermidis* (25 isolates).

CONCLUSIONS: MRSA colonization did not increase during longitudinal study. *S. aureus* colonization remained fairly stable throughout the study (17 – 26%). Species colonization with non *S. aureus* species (e.g. *S. hominis*, *S. epidermis, S. haemolyticus*) increased significantly (9.2 – 82.3%) during clinical rotations. Knowledge of infection control and compliance may have contributed to an absence of MRSA colonization; however, the colonization by other staphylococci has been shown to be a risk factor for MRSA acquisition.

ABBREVIATIONS: MRSA = Methicillin-resistant *Staphylococcus aureus*; CA-MRSA = Community-associated methicillin resistant *Staphylococcus aureus*; HA-MRSA = Healthcare-associated methicillin resistant *Staphylococcus aureus*; MSSA = methicillin-sensitive *Staphylococcus aureus*; CLS = Clinical Laboratory Science; OR = odds ratio; CI = Confidence Interval; HCWs = Healthcare Workers; HAIs = Healthcare Associated Infections

INDEX TERMS: Methicillin-resistant *Staphylococcus aureus*, Community acquired infections, Nursing research, Nosocomial infections, Carrier state

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Rodney E. Rohde, PhD, MS, SV, SM(ASCP)^{CM}MB^{CM}, *Texas State University, College of Health Professions, Clinical Laboratory Science, San Marcos, TX*

Tom Patterson, MS, BS, MT(ASCP), *Texas State University, College of Health Professions, Clinical Laboratory Science, San Marcos, TX*

Barbara Covington, PhD, RN, *Texas State University, College of Health Professions, St. David's School of Nursing, San Marcos, TX*

Bob Edward Vásquez, PhD, *Texas State University, College of Applied Arts, School of Criminal Justice, San Marcos, TX*

Gerald Redwine, MS, CLS (ASCP), *Texas State University, College of Health Professions, Clinical Laboratory Science, San Marcos, TX*

Emillio Carranco, MD, *Texas State University, Student Health Center, San Marcos, TX*

Address for Correspondence: Rodney E. Rohde, PhD, MS, SV, SM(ASCP)^{CM}MB^{CM}, *Chair & Professor, CLS, Associate Dean of Research, College of Health Professions, Texas State University, Clinical Laboratory Science, HPB 363, 601 University Drive, San Marcos, TX 78666-4616, 512-245-2562, rrohde@txstate.edu*

INTRODUCTION

According to the Centers for Disease Control, a leading cause of healthcare-associated infections (HAIs) is methicillin-resistant *Staphylococcus aureus* (MRSA). Infections with MRSA have surpassed HIV as a leading cause of morbidity and mortality in the United States.[1,2] It appears that the healthcare system may be a reservoir for MRSA and there is significant morbidity and mortality associated with HAIs.[3] Healthcare-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) is responsible for approximately 85% of invasive MRSA infections.[1]

In addition to HA-MRSA, this infection is also present in the community. Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains are responsible for an increasing number of serious infections in non-hospitalized, previously healthy young persons.[4] CA-MRSA has been shown to be easily transmissible in communal settings including prisons, schools, sport team areas (locker rooms, showers, wrestling mats, etc.).[4,5] Risk factors identified include: sharing of clothing, sports equipment, towels, razors, soap; improper care of skin trauma; crowded living conditions; lack of cleanliness and personal hygiene.[5-7] Studies indicate, however, the majority of MRSA infections are of the HA-MRSA type so it appears that healthcare settings pose the greatest threat to the general public.

Colonization by *S. aureus* of the nares has been identified as one risk factor for infection. An understanding of additional risk factors for transmission of *S. aureus* and MRSA is critical for identifying the potential for infection. Several studies have investigated the prevalence of nasal colonization in subpopulations of outpatient healthcare facilities, hospitals, jails, and intravenous users of non-prescribed drugs.[3,8-15] Some single point prevalence studies (studies with one sample collection) have investigated medical students[16-18] but very few studies of healthcare workers (HCWs) have examined longitudinal risk factors for *S. aureus* and HA-MRSA over a significant time period. This two year longitudinal study examined risk factors for acquisition of *Staphylococcus* and the length of time it takes for colonization of HCWs new to the healthcare field.

The study design assessed the initial carriage and infection rates in a cohort of new nursing students and followed these students throughout their clinical rotations in various healthcare settings over five semesters of clinical experiences with six times of measurement. All hospitals institute infection control precautions for patients with positive MRSA cultures and many now utilize a pre-admission MRSA detection method. This approach routinely does not include cultures of HCWs who are at risk for acquiring or transmitting this infection. The value of routine screening of HCWs who have direct patient contact for MRSA has not been thoroughly investigated.

An interim report (one year half-way point) of this study was published earlier.[19] Importantly, in this final report the authors document the staphylococcal variety and transition of species between the final two waves of collection. While it was surprising to observe an absence of MRSA isolates throughout the study, the colonization of nursing students by other species of *Staphylococcus* is clinically relevant because MRSA risk increases with this finding.

MATERIALS AND METHODS

Sample and Data Acquisition

A longitudinal (i.e., repeated measures over time) design was used to determine the rate of *S. aureus* and MRSA carriage in an incoming cohort of nursing students and to describe exposures (risk factors) associated with carriage. Explanation of the study was provided by investigators from the St. David's School of Nursing. To eliminate coercion, recruitment was accomplished by Clinical Laboratory Science (CLS) investigators. A purposive sampling strategy took place with the final sampling consisting of nursing students over the age of eighteen. All participation was voluntary, and prior to

participation, students completed informed consent through contact with CLS personnel. The Institutional Review Board (IRB) of Texas State University approved all procedures and protocols for this study (#2010F5693).

Investigators from two units of the College of Health Professions, St. David's School of Nursing and the Clinical Laboratory Science (CLS) Program along with the Student Health Center and a statistician from the College of Applied Arts (School of Criminal Justice) collaborated on the responsibilities of this longitudinal study. Reported are the complete six wave results from the enrollment baseline (Wave 1) through the five additional waves of this long-term study which differs from previous studies which examined risk factors for MRSA carriage rates at a single point in time in a cross-sectional format. Risk factors appearing on the questionnaire were determined by a collaborative effort by the investigators of this study. This investigation sought to identify both healthcare-associated and community-associated risk factors over a two year period in HCWs who were completing clinical rotations in acute healthcare settings.

Before each collection wave, nursing students were instructed by CLS investigators on the proper technique for collection of nasal swabs. All students were observed during the collection process. Any collection swab dropped or possibly contaminated in any way was disposed of and a new swab was used for collection. Nasal swabs were screened for *S. aureus* and MRSA using standard CLS protocols described previously.[12,13,19] A positive MRSA culture represented an end-point for participation in the study. For any presumptive positive MRSA result, a confirmatory sample was sent to a certified clinical laboratory. The principal investigator would then inform any participants of a positive culture and would offer medical intervention by the Medical Director of the Student Health Center. Treatment consisted of bactroban (mupirocin) for nasal decolonization and the oral antibiotic doxycycline.

Laboratory Analysis

Nasal swab specimens were screened for methicillin-sensitive *S. aureus*, (MSSA) S. "other," (staphylococcal species other than *aureus* which may be coagulase positive or negative) and MRSA using standard mannitol salt agar (MSA) and CHROMagar ™ MRSA screening agar (Becton Dickinson BBL, Franklin Lakes, NJ), Dry Spot Staphytect Plus test kits (Oxoid Limited, Lenexa, KS), and Dropit catalase reagent (Key Scientific Products, Round Rock, TX). Positive colony growth on CHROMagar was confirmed as MRSA by Vitek 2 (bioMerieux, Hazelwood, MO) susceptibility testing at Central Texas Medical Center (CTMC, San Marcos, TX) using Vitek GN19 susceptibility cards. Cards were inoculated and incubated in the Vitek 2 per manufacturer recommendations and results were analyzed by the advanced expert system, software version R04.03. All tests were performed according to manufacturer's instructions. All growth on MSA or CHROMagar not consistent with *S. aureus*, S. other or MRSA was discarded. *S. aureus*, MRSA, and *S. epidermidis* organisms were provided by CTMC, as confirmed by Vitek, and were used as positive and negative controls during all microbiological testing.

Data Screening and Analysis

Respondents were given the questionnaire shown in Figure 1 and investigators gathered and entered answers from the de-identified questionnaire into an Excel database (Microsoft, Redmond, WA). CLS investigators entered laboratory-related results into the database. The six waves of data were verified for completeness and accuracy and all data were then pooled (i.e., "stacked"). Because repeated measures are nested in individuals, the primary analytical issue was within-individual clustering. In other words, an individual's own measures are likely to be more similar to each other (i.e., clustered) than they are to measurements from another individual. This positive within-individual clustering produces downwardly-biased standard error estimates and inflated test statistics. The mixed-effects approach to analyzing longitudinal data has some advantages over traditional repeated measured analysis of variance, namely the increased flexibility in accommodating missing cases.[20]

An additional issue was the structure of the disturbance and the possibility that the within-individual clustering depended on time. In other words, data points closest in time were more correlated, which is consistent with autocorrelation in the form of a first-order autoregressive process, (i.e., an AR(1) error process). Due to the nested structure of the observations, serial correlation, and the binary-outcome dependent varia-

Age

Gender (Circle)	
Male	Female

Ethnicity (Circle)				
Hispanic	African-American	Caucasian	Asian	Other

INFECTIONS:

1	In the past 12 months, have you had a skin infection, boil, or sore?	Yes	No	Don't know/ Prefer not to answer
2	In the past 12 months, has a doctor told you that you have a skin infection called MRSA, "mersa," or antibiotic resistant Staph?	Yes	No	Don't know/ Prefer not to answer
3	Have you ever heard of MRSA, "mersa," or antibiotic resistant Staph?	Yes	No	Don't know/ Prefer not to answer

If so, how did you hear about it?

_____

HEALTHCARE

4	In the past 12 months, have you been a patient in the hospital?	Yes	No	Don't know/ Prefer not to answer
5	In the past 12 months, have you had surgery?	Yes	No	Don't know/ Prefer not to answer
6	In the past 12 months, have you worked in a healthcare facility?	Yes	No	Don't know/ Prefer not to answer
7	In the past 3 months, have you taken any antibiotics?	Yes	No	Don't know/ Prefer not to answer
8	In the past 12 months, have you used intravenous drugs?	Yes	No	Don't know/ Prefer not to answer

LIVING CONDITIONS

9	Are you currently living in a dorm?	Yes	No	Don't know/ Prefer not to answer
10	In the last 6 months, have you lived in a dorm?	Yes	No	Don't know/ Prefer not to answer
11	In the past 12 months, have you been in jail?	Yes	No	Don't know/ Prefer not to answer
12	In the past 12 months, have you participated in athletics?	Yes	No	Don't know/ Prefer not to answer

CLINICAL CARE EXPERIENCE- only answer for the semester you just completed.

13	During J1 semester, did you care for a patient who was on contact isolation for MRSA?	Yes	No	Don't know/ Prefer not to answer
14	During J2 semester, did you care for a patient who was on contact isolation for MRSA?	Yes	No	Don't know/ Prefer not to answer
15	During J3 semester, did you care for a patient who was on contact isolation for MRSA?	Yes	No	Don't know/ Prefer not to answer
16	During J4 semester, did you care for a patient who was on contact isolation for MRSA?	Yes	No	Don't know/ Prefer not to answer
17	During J5 semester, did you care for a patient who was on contact isolation for MRSA?	Yes	No	Don't know/ Prefer not to answer

Figure 1. Questionnaire for risk factors to *Staphylococcus aureus* and Methicillin resistant *Staphylococcus aureus* (MRSA).

bles, the investigators used generalized estimating equations (GEE) to obtain population-averaged panel logistic regression models.[21] The models which allow for an AR(1) process, were estimated with Stata version 13.

RESULTS

Study Population

The initial sample consisted of 87 nursing students over the age of eighteen, with the average at baseline of 24.5 years. Males represented 12.6% of the cohort while 74.7% were Caucasian. By Wave 6, only 62 of the original 87 respondents provided data. This attrition did not affect the sample composition in any meaningful way with respect to sex and race as the multivariate models include control variables for sex and race.

Measures

Clinical personnel provided survey instruments and tools for data collection, self-administration procedures were relied upon for data collection. The students themselves performed their own nasal swabs under supervision and completed the self-administered questionnaire. Nasal swab specimens were then screened for *S. aureus*, species other than *S. aureus*, and MRSA using standard CLS protocols previously described.[19] Table 1 shows the percent of the sample that tested positive for each category of infection across 6 waves of data collection. Only staphylococci isolated from Wave 5 and Wave 6 were speciated due to limited funds available for the study.

At baseline, one respondent tested positive for MRSA and no one thereafter had a positive MRSA culture. Similar to MRSA, the incidence of *S. aureus* appeared more frequently but was still relatively stable over time ranging between 17% and 26%. Compared to Wave 1, a 6% increase in *S. aureus* occurred in Wave 2, but the difference was not statistically significant. One item of special interest was the incidence of S. other in Wave 2 which increased dramatically and remained elevated throughout the rest of the study. By Wave 6, over 80% of the sample tested positive for S. other, whereas less than 10% tested positive at baseline. A difference in proportions test was used to compare the incidence of Wave 1 to the incidence of each subsequent wave. As is seen in Table 1 there is a significant increase in S. other infection over time. Ultimately a different estimation strategy was used to identify correlates of infection.

Risk Factors and Control Variables

One central focus of this study was whether exposure to the four measures or conditions discussed below represents risk factors for contracting *S. aureus*, *S.* other, and MRSA. The first measure was a dummy-coded item for exposure to a healthcare environment as a result of being a patient (1= yes). The measure was based on whether the respondent: (1) had been a patient in a hospital; (2) had surgery; (3) had taken antibiotics; and/or (4) had used intravenous drugs since the last interview. The second measure was also a dummy-coded item measuring the respondent's exposure to the healthcare environment (1 = yes), but in the capacity of a volunteer or worker (including a student worker). The third measure was whether the respondent had close contact with someone diagnosed with MRSA. The fourth measure was also a dummy-coded variable based on whether the respondent: (1) participated in athletics; and/or (2) used a workout center or gym since the last interview. In the analysis, investigators held constant the effects of age, sex (1 = male), and race of the respondent (White or not-White). Additionally, investigators held constant whether the subject reported having a skin infection, boil, or sore since the last interview. Table 2 provides the percent of the sample with exposure to healthcare settings as a patient, worker/volunteer, and the percent of the sample having contact with a person diagnosed with MRSA, and involvement in gym or sport activities across all 6 waves.

Table 3 shows the odds ratio and Z scores for the four different exposure conditions in addition to the control variables: age, male, white, and had a boil or skin infection since last interview. The first model explains the logit of a positive infection of *S. aureus*. The only variable in the model that has a statistically significant effect on this outcome is whether the respondents had a boil or skin infection since the last interview. Those with a boil or skin infection had almost triple the odds of an infection with *S. aureus* (OR = 2.94, $p < .01$). In the second model respondents with a work-related exposure to healthcare settings had more than 4 times the odds of infection with S. other compared to those without such exposure (OR= 4.41, $p < .001$). Involvement in sports activities or attending a gym or workout facility was also associated with a more than doubling of the odds of testing positive for S. other (OR= 2.45, $p < .01$).

Table 1. Percent of sample with positive nasal swab

By infection type and wave							Percent Change from Wave 1 to:				
	Wave 1	Wave 2	Wave 3	Wave 4	Wave 5	Wave 6	Wave 2	Wave 3	Wave 4	Wave 5	Wave 6
MRSA	1.2%	0.0%	0.0%	0.0%	0.0%	0.0%	NA	NA	NA	NA	NA
S. aureus	20.7%	26.4%	20.0%	21.4%	19.4%	17.7%	5.7	-0.7	0.7	-1.3	-2.9
S. other	9.2%	68.9%	80.0%	78.6%	77.6%	82.3%	59.7**	70.8**	69.4**	68.4**	73.1**

** = p < .001
Note: We suggest viewing these from a descriptive statistics perspective since the alpha error rate increases with multiple comparisons.

Table 2. Percent of sample with exposure to healthcare setting, people with MRSA, and gym/sports activities

By variable and wave	Wave 1	Wave 2	Wave 3	Wave 4	Wave 5	Wave 6
Exposure to healthcare settings						
As a patient	32.1%	39.2%	23.1%	27.1%	29.9%	29.0%
As a worker/volunteer	27.5%	78.5%	98.5%	94.3%	91.0%	90.3%
Contact with person diagnosed with MRSA	8.0%	16.8%	60.1%	48.6%	47.8%	47.5%
Involvement in gym/sports activities	32.1%	81.9%	79.4%	71.4%	61.2%	79.0%

*** = p < .001
Note: At baseline, the reference period is the previous 12 months. The investigators suggest viewing these from a descriptive statistics perspective since the alpha error rate increases with multiple comparisons.

Table 3. Logistic regression models explaining positive infection

By infection type	*S. aureus*		*S.* other	
	Odds Ratio	Z	Odds Ratio	Z
Exposure to healthcare settings				
As a patient	0.81	-0.83	0.90	-0.39
As a worker/volunteer	1.29	0.85	4.41**	5.03
Contact with person diagnosed with MRSA	0.94	-0.24	1.07	0.24
Involvement in gym/sports activities	1.08	0.28	2.45**	3.38
Control variables				
Age	0.99	-0.18	1.01	0.37
Male	1.59	0.81	0.75	-0.58
White	1.52	0.88	0.88	-0.36
Had boil/skin infection since last interview	2.94**	3.21	0.74	-0.77
Number of observations	364	364		
Number of subjects	69	69		
Model Wald (Chi-square)	12.19	48.95**		

* = p < .05
** = p < .01
*** = p < .001

Upon review of the interim study,[19] the investigators were asked by reviewers to provide speciation of all staphylococcal species if possible for Waves 5 and 6. Table 4 shows that speciation. Speciation of only the final two waves was possible due to limitations in the study budget. A large variety of *Staphylococcus* spp. and other bacteria was identified with a 72% change in species from Wave 5 to Wave 6. The most frequently occurring species at Wave 5 was *S. hominis* (21 isolates) while the most frequently occurring species at Wave 6

Table 4. Species identification of isolates with percent change of species from wave 5 to wave 6

Isolate	Wave 5	Wave 6	Species change
50	non-participant	*S. hominis*	NA
75	non-participant	*S. epidermidis*	NA
104	*S. hominis*	non-participant	NA
110	*S. epidermidis*	*S. epidermidis*	N
114	*S. epidermidis*	non-participant	NA
187	*S. aureus*	*S. aureus*	N
219	*S. auricularis/Leuconostoc mesenteroides*	*S. warneri*	Y
248	*S. hominis*	*S. capitis*	Y
254	non-participant	non-participant	NA
258	*L. mesenteroides*	*S. aureus*	Y
267	*S. hominis*	non-participant	NA
287	*S. aureus*	*S. epidermidis*	Y
296	*L. mesenteroides*	*S. capitis*	Y
299	non-participant	non-participant	NA
328	*S. epidermidis*	*Kocuria rosea*	Y
337	*S. auricularis* / *S. hominis* *Leuconostoc mesenteroides* / *S. hominis* *S. hominis*	*S. hominis*	Y
347	*S. hominis*	*S. epidermidis*	Y
350	*S. saprophyticus*	*S. lugdunensis*	Y
356	*S. cohnii*	*S. hominis*	Y
364	*S. capitis*	*Kocuria rosea*	Y
366	*S. hominis*	*S. capitis*	N
370	non-participant	non-participant	NA
373	non-participant	non-participant	NA
383	*L. mesenteroides*	non-participant	NA
389	*L. mesenteroides*	*S. epidermidis*	Y
398	*S. aureus*	*Kocuria kristinae*	Y
404	*S. aureus*	*S. aureus*	N
412	*S. gallinarum*	*S. aureus*	N
414	non-participant	*Kocuria rosea*	Y
424	*S. aureus*	*S. hominis*	NA
427	*S. hominis*	non-participant	NA
431	*S. aureus*	*S. hominis*	N
433	*S. hominis*	*S. aureus*	N
446	non-participant	*S. epidermidis*	Y
450	*S. hominis*	*Kocuria rosea*	NA
452	*S. hominis*	non-participant	NA
460	*S. warneri*	*S. epidermidis*	Y
462	non-participant	*Kocuria rosea*	Y
469	*S. hominis*	*S. epidermidis*	NA
479	*S. epidermidis*	*S. epidermidis*	Y
481	non-participant	*S. aureus*	Y
485	*S. hominis*	non-participant	NA
491	*S. hominis*	*Kocuria rosea*	Y
495	*S. aureus*	non-participant	NA
504	non-participant	*S. epidermidis*	Y
508	*L. mesenteroides*	non-participant	NA
523	*S. capitis*	*S. epidermidis*	Y
537	*S. epidermidis*	*S. lugdunensis*	Y
546	*L. mesenteroides*	*S. aureus*	Y
552	*S. epidermidis*	*S. epidermidis*	Y

Table 4. (Continued)

Isolate	Wave 5	Wave 6	Species change
556	non-participant	*S. hominis*	Y
558	*S. aureus*	non-participant	NA
568	*S. aureus*	*S. haemolyticus*	Y
571	*S. hominis*	*S. aureus*	N
581	*S. hominis*	*S. hominis*	N
594	*S. epidermidis*	*S. epidermidis*	Y
614	No growth/Neg	S. *epidermidis*	N
619	*S. epidermidis*	non-participant	NA
635	*S. aureus*	*S. epidermidis*	N
640	non-participant	*S. aureus*	N
642	non-participant	non-participant	NA
645	non-participant	non-participant	NA
662	non-participant	non-participant	NA
664	No growth/Neg	non-participant	NA
671	*L. mesenteroides*	*S. epidermidis*	NA
690	*S. aureus*	non-participant	NA
693	*S. hominis*	*S. aureus*	N
702	*S. epidermidis*	non-participant	NA
716	non-participant	*S. epidermidis*	N
738	*S. hominis*	non-participant	NA
767	non-participant	*S. hominis*	N
773	non-participant	*S. epidermidis*	NA
787	non-participant	*S. epidermidis*	NA
796	*S. capitis*	non-participant	NA
802	*S. aureus*	*S. epidermidis*	Y
806	*S. epidermidis*	*S. aureus*	Y
808	*S. aureus*	*S. saprophyticus*	Y
841	non-participant	*S. epidermidis*	Y
844	non-participant	*S. epidermidis*	NA
861	*S. hominis*	non-participant	NA
879	*S. epidermidis*	non-participant	NA
885	*S. haemolyticus*	non-participant	NA
892	*S. epidermidis*	*S. hominis*	Y
895	*S. hominis*	*S. warneri*	Y
914	*S. hominis*	*Kocuria rosea*	Y
931	*L. mesenteroides*	*S. epidermidis*	Y
972	*S. hominis*	*S. epidermidis*	Y
986	*S. epidermidis*	*S. haemolyticus*	Y
997		*S. epidermidis*	N
Total = 89	Total = 65 isolates	Total = 63 isolates	Percent change = 72%

*NA = not applicable; N = no; Y = yes
Note: Percent change was determined by total Y (39) / total N + Y (54) x 100

was *S. epidermidis* (25 isolates). This suggests that the composition of the microbial flora was dynamically changing over time.

DISCUSSION

Since HCWs such as phlebotomists, nurses, and physicians, work at the intersection between a wide range of different types of healthcare facilities and the community (schools, athletic facilities, prisons, universities, etc.), they may serve as reservoirs and vectors allowing for cross-transmission of MRSA, *S. aureus*, and or other multiple drug resistant organisms.[14] With this in mind, HCWs have been extensively studied in many reports with regard to sporadic, epidemic, and endemic MRSA; however, most of these studies were snapshots usually limited to outbreak reports.[22] The investigators of this report set out to follow a cohort of nursing students throughout their

clinical rotations in anticipation of investigating how staphylococci, particularly *S. aureus* and MRSA, colonize (or do not colonize) new HCWs and the nature of their conversion to a carrier state. Most current studies have been point prevalence studies that have not looked at long term monitoring of HCWs. By identifying and documenting risks associated with their daily activities and lifestyles, this report shed light on factors that contribute to colonization of HCWs with *S. aureus*, MRSA and even other non-*S. aureus* species. The knowledge, learning, and overall understanding about HAIs, like MRSA, have been documented previously[23] as an important process for improved infection control and behavior change in the general public. This study supports those findings as critically important for HCWs too in regard to self-directed and sometimes transformational processes in their approach to infection control compliance.

The incidence of *S. aureus* in the nursing student cohort was found to be slightly lower (17 – 26%) but similar to previously reported studies that show a range of 10% to 37% carrier state.[12,13,15-17] Another study found that age, gender, chronic sinusitis, medical student status, and hospitalization were associated with carrier status for *S. aureus*[15] which this study did not demonstrate. Surprisingly, this study found hospitalization was not a risk factor which is the opposite of the findings of a previous study by Rohde, Denham, and Brannon.[13] This study supported previous studies that showed time spent in a hospital as a volunteer or worker did pose an increased risk. Finally, nursing students reporting a boil or skin infection during their clinical rotations were almost three times (OR = 2.94, $p < .01$) more likely to be colonized by *S. aureus* compared to students who did not report skin infections.

MRSA colonization in this nursing student cohort did not increase while colonization with *S. aureus* remained stable. It can be argued that these results may be due to increased awareness of the students due to their concurrent education in infectious disease control. At the outset of the study, the nursing faculty conducted an orientation on MRSA and its risk to the healthcare environment and to the community. This orientation may have contributed to strict compliance with infection control procedures and the use of barrier precautions (hand washing, personal protective equipment, isolation procedures, etc.). Nursing students often reported "awareness" of MRSA patients in isolation and the subsequent adherence to hygiene and other isolation procedures. Students may also be motivated to strictly follow compliance protocols as they know they are being observed and graded during their clinical rotations, as well as follow-up nasal swabs conducted during the study.

Several risk factors may account for recent increases in MRSA infection such as the over-prescription of antibiotics, prevalence of HAIs in the healthcare environment, gradual emergence of resistant virulent strains, and transmission vectors in a healthcare setting (hospital, clinic, retirement home, etc.).[24] National mandates and congressional concern over MRSA and other HAIs have emerged recently due to the high financial and human costs associated with these infections. Hospitals are being asked to develop more aggressive protocols to decrease HAIs such as MRSA colonization and infection.[25] There are several approaches being followed including the use of the MRSA prevention bundle, a five step process of training staff to identify, isolate, and treat infected patients thereby reducing surgical site infections.[26] In most infection control approaches, education and emphasis on strict compliance play a critical role in the decrease of MRSA colonization and infection. The investigators of this study believe that initial orientation and continued focus on hygiene, fomites, reservoirs and prevention minded thinking had a significant impact on the prevention of colonization of the nursing student cohort.

An unexpected finding of this study is the nasal colonization of students with staphylococcal species other than *S. aureus* as shown in Table 4 (e.g. *S. epidermidis, S. hominis, S. haemolyticus*, etc.). The prevalence of S. other (species other than *aureus*) in this study increased very quickly. The investigators chose to use "S. other" in this study instead of coagulase negative staphylococci (CNS) because there are several species other than *S. aureus* that can be coagulase positive (e.g. *S. delphini, S. hyicus, S. intermedius, S. lutrae, S. pseudointermedius, S. schleferi* subsp. *coagulans* and *S. leei*). By the sixth wave of data collection, 82% of participants tested positive for *S.* other whereas only 10% were positive for *S.* other at baseline. There was a dramatic difference in species detected of *S.* other from Wave 5 to Wave 6 in 72% of the swabs. The most

frequently occurring species difference was *S. hominis* versus *S. epidermidis*. This species difference occurred in seven isolates, which represent about 18 percent of the difference from Wave 5 to Wave 6. Due to financial constraints, only staphylococci isolated in Wave 5 and Wave 6 were identified by individual species. The high percentage and diversity of microbial species was an unexpected finding leading the investigators to believe HCW nasal flora may be transient or persistent. The meaning or importance of this finding is unclear. Further studies with additional time points of collection are required to investigate the possible causes of nasal microbial flora change and what, if any, healthcare and personal behavior or environmental factors may influence this phenomenon. It is not certain that one would find an absence of MRSA in a HCW if nasal swabs were conducted unannounced. In this study, compliance awareness and knowledge of MRSA was apparent throughout the study because of the nature of the experimental design.

Participants who worked or volunteered in a healthcare facility were more than four times as likely (OR = 4.41, p <.01) to be colonized with S. other, while those who were involved in sports or gym activities were two and one half times (OR = 2.45, p < .01) as likely to be colonized. Although several studies have shown that being involved in healthcare and or athletics are risk factors for MRSA and or *S. aureus*,[2-4,7,12,13,15] it has rarely or has never been reported that these are significant risk factors associated with colonization of species other than *S. aureus*. The investigators hypothesize that this may be explained simply by more participants using indoor work-out facilities (gyms, weight equipment, and cardio equipment) more during the winter versus the summer when the initial specimens were collected. It is not well understood if this phenomenon may play a role in nosocomial transmission. Perhaps colonization with other staphylococci species may play a protective role by competitive inhibition of other pathogens in nasal colonization, or conversely, it may play a role in HAIs that have not been documented. The investigators will follow this unexpected finding in future studies and anticipate gaining additional insight by determining the types of different staphylococci present, as well as persistent versus transient carriers of staphylococcal species.

CONCLUSION

MRSA, along with other HAIs, has emerged as a growing world-wide problem in the past few decades. Common-sense approaches to prevention, along with intelligent use of the laboratory (culture of wounds, antibiotic susceptibility, etc.) as well as proper decontamination of the healthcare environment and available, correctly identified antimicrobials can protect individuals from this growing threat. Healthcare officials, community leaders, and public health policy makers should be aware of the potential for transmission risk and outbreak scenarios that could develop in the environment of HCW populations and their daily work-related tasks. While MRSA was not documented in this study, the finding of other *Staphylococcus* spp. along with others referenced, illustrates the growing importance of patient and community education and where it intersects with compliance of basic infection control prevention efforts. Indeed, this study may have shown that knowledge and awareness of HAIs, such as MRSA, by nursing students, contributed to an absence of MRSA colonization. The HCW and healthcare environment, as well as the general public, is understudied with respect to the prevalence of HAIs, like MRSA, over time and in terms of persistent versus transient colonization of these resistant microbes. Further research is also needed in the area of knowledge, awareness, and the learning needs (gaps in knowledge) of the general public with respect to MRSA and other antibiotic resistant organisms.[23]

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