

Methicillin Resistant *Staphylococcus aureus* (MRSA): An Interim Report of Carriage and Conversion Rates in Nursing Students

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OBJECTIVE: To evaluate and characterize MRSA and staphylococci carriage and conversion rates in nursing students across clinical semester rotations and to describe risk factors.

DESIGN: A prospective, longitudinal cohort design (interim report) with three times of measurement. Data collected between August 2010 and May 2011 (ongoing longitudinal study 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 represented an end-point for a participant. Intervention offered was bactroban (mupirocin) for nasal decolonization and an oral antibiotic, doxycycline; post-treatment collection 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 collected demographics and risk factors. Generalized estimating equations calculated population-averaged panel logistic regression models allowing for an AR(1) error by Stata version 12.

RESULTS: MRSA colonization did not increase. *S. aureus* prevalence (20-26%). Species prevalence other than *S. aureus* increased (9.2% to 80%). The following associations were found to be statistically significant: boil or skin infection odds with *S. aureus* (OR= 2.43, $p < .05$), working or volunteering in healthcare facility odds with *S. other* (OR= 2.72, $p < .05$) and gym and

sports activities odds with *S. other* (OR= 4.98, $p < .001$).

CONCLUSIONS: MRSA colonization did not increase. Knowledge and understanding of MRSA (risks) may play a role in compliance and barrier precautions. *S. aureus* colonization remained stable (25-30%). Species colonization other than *S. aureus* (e.g. *S. epidermis*, *S. haemolyticus*) increased to significant levels.

ABBREVIATIONS: MRSA= Methicillin resistant *Staphylococcus aureus*; CA-MRSA=Community-associated methicillin resistant *Staphylococcus aureus*; HA-MRSA=Healthcare-associated methicillin resistant *Staphylococcus aureus*; CLS = Clinical Laboratory Science; OR = Odds Ratio; CI = Confidence Interval; HCWs = Healthcare Workers; Healthcare associated infections = HAIs.

INDEX TERMS: Methicillin resistant *Staphylococcus aureus*, MRSA, Community acquired infections, Nursing research, Nosocomial infections, HAIs.

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INTRODUCTION

Healthcare associated infections have become one of the most costly and deadly growing public health threats of our time. The Centers for Disease Control and other studies estimate that Methicillin Resistant *Staphylococcus aureus* (MRSA) has surpassed HIV as the leading cause of morbidity and mortality in the U.S.^{1,2} For the past several decades, MRSA has become a serious problem in patients with exposure to the healthcare system, and is responsible for substantial morbidity and mortality in hospitals around the world.³ Today, healthcare-associated methicillin resistant *Staphylococcus aureus* (HA-MRSA) accounts for approximately 85% of all invasive MRSA infections.¹

MRSA has also evolved in the community and is unrelated to the evolution of HA-MRSA in hospitals and other settings. These community associated strains, known as community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) have been causing an increasing number of serious infections in non-hospitalized, previous healthy young people.⁴ CA-MRSA is easily transmissible, not only between families, but also in larger close-contact communal settings such as prisons, schools, and sports team environments (e.g. locker rooms, wrestling mats, etc.).^{4,5} Environmental sources, such as the sharing of clothing, sports equipment, towels, razors, and soaps; improper care of skin trauma; crowded living conditions, along with lack of cleanliness and personal hygiene, are identified as possible risk factors for both CA-MRSA and HA-MRSA infections.⁵⁻⁷ Regardless of the type of MRSA, the risk of MRSA carriage by healthcare workers (HCWs) poses a real threat to the healthcare environment as well as the general public.

Because nasal carriage (colonization) of *S. aureus* has

been identified as a major risk factor for subsequent infections, an understanding of the risk factors for carriage of *S. aureus* and MRSA is crucial to understanding the potential for transmission and invasive infections. A variety of studies have examined community prevalence of nasal carriage in subpopulations including hospitals, outpatient settings, jails, and injection drug users.^{3,8-15} Several point prevalence studies have been conducted with medical students¹⁶⁻¹⁸ but very few, if any, longitudinal studies have examined the characteristics of *S. aureus* and MRSA in a population of HCWs. This project seeks to add to the knowledge of risk for acquisition as well as length of time to colonization of healthcare professionals.

The purpose of this research was to assess initial prevalence or acquisition of *S. aureus* or MRSA in a cohort of nursing students and to follow these students over five semesters of clinical care experiences. Hospitals have instituted specific contact precautions for patients who have a known positive nasal swab test for MRSA in the acute care setting. This care has not to this point included routine examination of healthcare workers for risk either to acquire this bacterium or to contribute to its spread. The question of screening for MRSA in HCWs remains understudied.

It was anticipated that the findings of this study would be utilized to (1) examine risk factors associated with conversion to MRSA colonization, (2) develop a future study examining prevalence of MRSA in healthcare workers from the public domain, (3) provide preliminary data to develop a pilot program to improve control and prevention of transmission of MRSA with respect to risk factors identified in this study, and (4) provide supportive data to institute an examination of compliance with contact isolation used in a local acute care setting including nursing attitudes toward enforcement, and patient comprehension of MRSA and contact isolation.

MATERIALS AND METHODS

Sample and Data Acquisition

A longitudinal (time-series) design was planned 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. Initial explanation of the study procedures was provided by the

investigators from the School of Nursing. To reduce potential coercion, recruitment was accomplished by Clinical Laboratory Science (CLS) investigators. A purposive sampling strategy took place with the final sample consisting of nursing students over the age of eighteen. All participation was voluntary, and prior to participation, students authorized informed consent via the CLS personnel. The Institutional Review Board (IRB) of Texas State University–San Marcos approved all procedures and protocols for this study (#2010F5693).

Investigators from two units of the College of Health Professions; School of Nursing and Clinical Laboratory Science (CLS) and the Student Health Center along with a statistician from the College of Applied Arts (Criminal Justice Department) collaborated on the responsibilities of this longitudinal study. Reported here are the interim results from enrollment/baseline and two additional data collection periods (waves) – one at the conclusion of the Junior 1 semester and one at the conclusion of the Junior 2 semester. While many studies have examined risk factors associated with carriage rates for MRSA in a cross-sectional format, this study sought to examine risk factors over time.

Nasal swab specimens were screened for *S. aureus* and MRSA using the standard CLS protocols and described previously.^{12,13} A positive MRSA swab represented an end-point for a participant. It was planned that the principal investigator would inform any participants of a positive result and that medical intervention would be offered to students by the Medical Director of the Student Health Center after a confirmatory swab was sent for testing to a certified clinical laboratory for confirmation. Treatment offered consisted of bactroban (mupirocin) for nasal decolonization and the oral antibiotic doxycycline. A swab was also planned for collection post-treatment to verify a negative nasal swab prior to the beginning of the next clinical rotation.

Laboratory Analysis

Nasal swab specimens were screened for *S. aureus*, *S. "other,"* (staphylococcal species other than *aureus* which may be coagulase positive or negative) and MRSA using the standard media mannitol salt agar (MSA) and CHROMagar™ MRSA screening agar (Becton Dickinson BBL, Franklin Lakes, NJ), Dry Spot Staphytest 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 (bioMérieux, Hazelwood, MO) susceptibility testing at CTMC (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 the 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 Central Texas Medical Center (CTMC) as confirmed by Vitek 2 analysis and were used as positive and negative controls during inoculation of all microbiological testing.

Data Screening and Analysis

Nursing investigators entered questionnaire (Figure 1) results and CLS investigators entered laboratory results into an Excel database (Microsoft, Redmond, WA) for initial data collection. Each wave of data was verified for completeness and accuracy, and data were then pooled. Because repeated measures were nested in individuals, the primary analytical issue was within-individual clustering. In other words, an individual's own measures were likely to be more similar to each other (i.e., clustered) than they were to measurements from another individual. This positive within-individual clustering produced downwardly-biased standard error estimates and inflated test statistics. As noted elsewhere, a mixed-effects approach to analyzing longitudinal data has some key advantages over traditional repeated measures analysis of variance.¹⁹ A multilevel approach allowed for this clustering, and had more flexibility in accommodating missing cases than repeated measures analysis of variance.

An additional issue, however, 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 variables, the inves-

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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).

tigators used generalized estimating equations (GEE) to obtain population-averaged panel logistic regression models.²⁰ We also allow for an AR(1) process. Overall, analysis was conducted using Stata version 12.

RESULTS – INTERIM REPORT

Study Population

The sample consisted of 87 nursing students over the age of eighteen, with the average age at baseline of 24.5 years. Males represented 12.6 % of the sample with 74.7% of the sample Caucasian. By wave 3, 70 of the original 87 respondents provided data. The investigators found that with respect to sex and race however, attrition had not affected sample composition in any meaningful way. Furthermore, because this study will continue, we expect to recover participants in the next wave of data collection. Table 1 shows the percent of the sample testing positive for each infection across our 3 waves of data collection.

At baseline, only one respondent tested positive for MRSA. No one since has had a positive MRSA nasal swab. Similar to MRSA, the prevalence of *S. aureus* was also relatively stable over time, ranging between 20 and 26 percent. Compared to wave 1, it was observed that a 6% increase in *S. aureus* at wave 2 occurred, but this difference was not statistically significant. The prevalence of *S. other* in this sample, however, increased steadily and relatively quickly. By the third wave of data collection, 80% of the sample tested positive for *S. other*, whereas less than 10% tested positive at baseline. For completeness, a difference in proportions test was utilized to compare wave 1 to wave 2 and wave 3. As shown in Table 1, it was shown that a statistically significant increase in *S. other* infection occurred over time. However, we ultimately used a different estimation strategy to identify correlates of infection.

Table 1. Percent of sample with positive nasal swab

	Wave 1	Wave 2	Wave 3	Wave 1 to Wave 2		Wave 1 to Wave 3	
				Percent Change	Z	Percent Change	Z
MRSA	1.15%	0.0%	0.0%	NA	NA	NA	NA
<i>S. aureus</i>	20.7%	26.4%	20.0%	5.7	0.89	-0.7	-0.11
<i>S. other</i>	9.2%	68.9%	80.0%	59.7***	8.97	70.8***	8.97

*** = p < .001

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

Risk Factors and Control Variables

The central focus of this study was concerned with whether exposure to the following four conditions represented 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 answering yes to any of four relevant items; the questionnaire asked respondents whether they had (1) been a patient in a hospital; (2) had surgery; (3) taken antibiotics; or (4) used intravenous drugs since their last interview. Table 2 provides the percent of sample with exposure across waves.

students.) Our third measure was whether the respondent had had close contact with someone diagnosed with MRSA since the last interview (1 = yes).

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
Exposure to healthcare settings			
As a patient	32.1%	39.2%	23.1%
As a worker/volunteer	27.5%	78.5%	98.5%
Contact with person diagnosed with MRSA	8.0%	16.8%	60.1%
Involvement in gym/sports activities	32.1%	81.9%	79.4%

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

The second measure was a dummy coded item also measuring exposure to a healthcare environment, but based on whether the respondent had worked or volunteered in a healthcare facility (1 = yes). (All students had spent a similar amount of time as nursing

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The fourth measure was also a dummy coded variable based on the following two items: whether the respondent: (1) participated in athletics; or (2) used a workout center since the last interview. Such activities might independently increase the risk of contracting *S. aureus*, *S. other*, and MRSA. We also collected general demographic information. In our analyses, we controlled for age, sex (1 = male), and race of the respondent (White or not-White). Additionally, we held constant whether the subject reported having a skin infection, boil, or sore since the last interview.

The first model, which explained *S. aureus*, was a relatively poor fit to the data. We observed that those who reported a boil or skin infection, compared to those who did not, had more than twice the odds of having *S. aureus*. However, the model chi-square test indicated no significant improvement over an empty model (8.08, $p = .42$). Results from the second model, which predicted testing positive for *S. other*, showed two substantive findings. First, those who worked or volunteered in a healthcare facility had almost 3 times the odds of having *S. other*, which was a statistically significant difference (OR = 2.72, $p < .05$). Second, those who reported being involved in gym and sports activities had about 5 times the odds of having *S. other*, and this difference too was significant (OR = 4.98, $p < .001$). Results are shown in Table 3.

DISCUSSION

Since HCWs, such as nurses, work at the intersection between hospitals, healthcare facilities (e.g. dialysis units and long-term care), and nursing homes on the one hand and interact with the community (schools, athletic facilities, prisons, universities, etc.) on the other, they may serve as reservoirs, vectors, or victims of MRSA, *S. aureus*, and/or other multiple drug resistant organisms' cross-transmission.¹⁴ In this respect, HCWs have been studied extensively in a variety of individual reports with regard to sporadic, epidemic, and endemic MRSA; however, the only previous review was limited to outbreak reports.²¹ The investigators of this interim report set out to follow a cohort of nursing students in anticipation of exploring how staphylococci, particularly *S. aureus* and MRSA, colonize HCWs (or do not colonize) and if there is a conversion longitudinally. Most current studies and reviews of staphylococci colonization or conversion have been point prevalence studies. By documenting risks associated with the daily

working routines and lifestyles of this population, this interim report highlights the findings at the halfway point of the study.

Table 3. Logistic regression models explaining positive infection

By infection type	<i>S. aureus</i>		<i>S. other</i>	
	Odds Ratio	Z	Odds Ratio	Z
Exposure to healthcare settings				
As a patient	0.80	-0.68	1.15	0.41
As a worker/volunteer	1.42	1.06	2.72**	2.95
Contact with person diagnosed with MRSA	0.86	-0.49	0.77	-0.77
Involvement in gym/sports activities	1.45	1.13	4.98***	4.43
Control variables				
Age	0.99	-0.25	1.01	0.31
Male	1.13	0.19	1.40	0.66
White	1.25	0.43	1.08	0.20
Had boil/skin infection since last interview	2.43*	2.14	1.76	1.12
Number of observations		233	233	
Number of subjects		83	83	
Model Wald (Chi-square)		8.08	41.75***	

* = $p < .05$

** = $p < .01$

*** = $p < .001$

The prevalence of *S. aureus* in our cohort of nursing students was slightly lower (20-26%) but similar to previously reported studies of 19% to 37%.^{12,13,15-17} Additionally, other investigators 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 diametrically opposed findings from those of Rohde, Denham and Brannon¹³ in that hospitalization was not related to carrier risk, but did replicate the results of previous studies that time spent in the hospital setting as either a volunteer or healthcare worker increased this risk. Lastly, nursing students reporting a boil or skin infection during a clinical experience were almost two and a half times more likely to be colonized with *S. aureus* (OR= 2.43, $p < .05$). Skin infections have been consistently reported as a major risk factor for both MRSA and *S. aureus* colonization and infections.¹²⁻¹⁴

MRSA colonization in this nursing student cohort population did not increase and, as mentioned above, *S.*

aureus colonization remained relatively stable. These results may have occurred because healthcare students were sensitized to risks of MRSA. At the outset of the study, nursing faculty conducted an orientation on what exactly MRSA represented and its risks in the healthcare and community environment. The orientation session may have influenced the student cohort resulting in increased compliance with use of barrier precautions (hand washing, personal protective equipment, patient handling, etc.). Indeed, nursing participants often reported “awareness” of quarantined MRSA patients and their subsequent adherence to hygiene and other infection control precautions. Many risk factors account for the increase in MRSA infections such as the over-prescription of antibiotics, the emergence of new virulent strains, and healthcare-setting transmission.²³ National mandates and congressional concern have erupted recently due to MRSA’s high financial and human costs, not to mention other HAIs. Hospitals are being asked to develop aggressive protocols to decrease HAIs such as MRSA colonization and infection.²⁴ There are many approaches being followed including the use of MRSA prevention bundles in surgical site infections.²⁵ In almost all approaches, education and compliance play a part of a critical, if not the singular important role, in the decrease of MRSA colonization and infection. The investigators of this study believe that our initial orientation and continued focus on hygiene, fomites, reservoirs, and prevention-minded thinking had an impact on the interim MRSA colonization of our nursing students remaining at a zero level.

An unexpected finding at the interim point of this investigation is the nasal colonization of participants with species other than *S. aureus* (e.g. *S. epidermidis*, *S. intermedius*, *S. haemolyticus*, etc.). The prevalence of *S. other* (species other than aureus) in this sample increased steadily and relatively quickly. The investigators chose to use “*S. other*” in this study instead of coagulase negative staphylococci (CNS) because there are species other than *S. aureus* that can be coagulase positive (e.g. *S. delphini*, *S. hyicus*, *S. intermedius*, *S. lutrae*, *S. pseudintermedius*, *S. schleiferi* subsp. *Coagulans* and *S. leei*) as well as the CNS. By the third wave of data collection, 80% of the sample tested positive for *S. other*, whereas less than 10% tested positive at baseline. Participants who worked or volunteered in a healthcare facility had almost three times the odds of having *S. other*, while those who

reported being involved in gym and sports activities had about five times the odds of having *S. other*, and this difference too was significant (OR = 4.98, $p < .001$). While other studies have shown that being involved with a healthcare facility and athletics are risk factors for MRSA and/or *S. aureus*,^{2-4,7,12,13,15} it has rarely or never been reported with species other than *S. aureus* nor has the rapid increase in prevalence. The investigators hypothesize that this may be explained simply by more participants using indoor workout facilities (e.g. gyms, recreational centers, etc.) more during the winter versus the summer when specimens were first collected. It is not well-understood if this phenomenon may play an important role in nosocomial transmission understanding. Perhaps, colonization with other staphylococci species may play a protective role by competitive inhibition of other pathogens in nasal colonization, or conversely, perhaps it may play a role in HAIs that have not been documented. The investigators will follow this unexpected finding through the completion of the study and anticipate gaining additional insight by determining the types of different staphylococci species present.

CONCLUSION

MRSA, along with other HAIs, has emerged as a growing world-wide problem in the past decade(s). Common-sense approaches to prevention, along with intelligent use of the laboratory (culture of wounds, antibiotic susceptibility testing, etc.) and available antimicrobials can protect individuals from this new threat. Healthcare officials, community leaders, and public health policy-makers should be aware of the potential transmission risk and outbreak scenario that could develop in the rich environment of HCW populations and their daily work-related tasks. This study, 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. Finally, research is desperately needed in the area of knowledge, awareness, and the learning needs (gaps in knowledge) of the general public with respect for MRSA and other antibiotic resistant organisms.²²

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