Comparative Medicine
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Vol 69, No 4
August 2019
Pages 311–320

Original Research

Carriage of Methicillin-resistant *Staphylococcus aureus* in a Colony of Rhesus (*Macaca mulatta*) and Cynomolgus (*Macaca fascicularis*) Macaques

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Methicillin-resistant *Staphylococcus aureus* (MRSA) carriage and infection are well documented in the human and veterinary literature; however only limited information is available regarding MRSA carriage and infection in laboratory NHP populations. The objective of this study was to characterize MRSA carriage in a representative research colony of rhesus and cynomolgus macaques through a cross-sectional analysis of 300 animals. MRSA carriage was determined by using nasal culture. Demographic characteristics of carriers and noncarriers were compared to determine factors linked to increased risk of carriage, and MRSA isolates were analyzed to determine antimicrobial susceptibility patterns, staphylococcal chromosome cassette mec (SCCmec) type, and multilocus sequence type (ST). Culture results demonstrated MRSA carriage in 6.3% of the study population. Animals with greater numbers of veterinary or experimental interventions including antibiotic administration, steroid administration, dental procedures, and surgery were more likely to carry MRSA. Susceptibility results indicated that MRSA isolates were resistant to β-lactams, and all isolates were resistant to between 1 and 4 nonβ-lactam antibiotics. In addition, 73.7% of MRSA isolates were identified as ST188-SCCmec IV, an isolate previously observed in an unrelated population of macaques and 15.8% were ST3268-SCCmec V, which has only been described in macaques. A single isolate had a novel sequence type, ST3478, and carried SCCmec V. These results suggest that NHP-adapted strains of MRSA exist and highlight the emergence of antimicrobial resistance in laboratory NHP populations.

Abbreviations: ACME, arginine catabolic mobile element; CA-MRSA, community-associated MRSA; CoNS, coagulase-negative staphylococcal species; MLST, multilocus sequence typing; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; PVL, Panton–Valentine leucocidin; SCCmec, staphylococcal chromosome cassette mec; TMS, trimethoprim–sulfamethoxazole

DOI: 10.30802/AALAS-CM-18-000089

Laboratory NHP have many of the same risk factors that predispose the hospitalized human population to infection with methicillin-resistant *Staphylococcus aureus* (MRSA), including a high density of individuals, use of indwelling devices, frequent antibiotic use, and immunosuppression.[8,34] MRSA carriage substantially raises the risk of MRSA infection in humans,[19,38] and knowledge of carrier status may prompt steps to minimize the risk of transmission and infection. However, little information regarding the prevalence, risk factors, and strains associated with MRSA carriage in populations of laboratory macaques is available to guide clinical decision making.

The emergence of MRSA has been widely documented in human and animal populations. *S. aureus* is part of the normal flora present on the skin and mucosal surfaces of humans, NHP, and livestock species but has the potential to cause a variety of infections, including skin and soft tissue infections, endocarditis, pneumonia, and sepsis.[41] Distinct lineages of *S. aureus* are often differentiated by multilocus sequence types (ST) that are determined according to the sequences of 7 core genes in the genome. MRSA strains arise through acquisition of *mecA* or, less commonly, *mecC*, which is carried on the mobile genetic element SCCmec that integrates site-specifically into the genome. *mecA* and *mecC* encode an acquired penicillin-binding protein, PBP2a, with low affinity for all β-lactam antibiotics, thereby conferring antimicrobial resistance. Frequently MRSA strains, especially those associated with the healthcare setting, also have resistance to nonβ-lactam classes of antibiotics, including lincosamides, macrolides, aminoglycosides, and fluoroquinolones. Because SCCmec is horizontally acquired, the same ST can be found among both MRSA and methicillin-susceptible *S. aureus* (MSSA) strains. However, SCCmec has entered into a limited number of ST, suggesting that restriction to uptake or integration exists among different lineages of *S. aureus*. MRSA strains are not necessarily more virulent than MSSA, given that each strain carries a unique set of virulence traits and resistance genes that define its potential for transmission, disease severity, and options for therapy.

MRSA was initially described as a nosocomial infection, becoming pervasive in the hospitalized human population during

Received: 14 Aug 2018. Revision requested: 13 Oct 2018. Accepted: 20 Jan 2019.
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the late 1970s and early 1980s, with the most recent large-scale analysis of US hospitals indicating a total carriage rate of 6.64% among inpatients.[14] However, this rate varies considerably between the populations studied, ranging from 3.8% of inpatients in a children's hospital in 2010[32] to 24.4% of ICU patients in a 2008 survey.[27] This carrier status is important because, although MRSA can survive in the environment for limited periods of time, it is susceptible to most commonly used disinfectants. Transmission is most frequently through direct contact with infected or colonized persons, with colonized patients serving as the main reservoir of *S. aureus* in healthcare facilities.[38]

NHP share many predisposing factors with humans, including natural nasal carriage of *S. aureus*.[37] In a previous study of 731 laboratory rhesus macaques, 39% had *S. aureus* isolated from the nasal passages, comparable to the approximately 35% of the human population that carries the bacteria commensally in the nares.[19] In a study of 596 laboratory macaques, MRSA was isolated from 17.6%.[31] Multiple case reports have presented the effects of MRSA infection in previously healthy macaques, including pneumonia[26] and necrotizing stomatitis,[20] and in association with risk factors that predispose NHP to opportunistic infections, including immunosuppression,[3,17,19] cranial implants,[15] and chronic, exteriorized catheters.[35] A population-based study in laboratory chimpanzees revealed a MRSA carriage rate of 69%, with isolates identified as primarily human community-associated (CA) MRSA strains.[11]

MRSA carriage in laboratory NHP is problematic from both the perspective of preventing research interference and from the risk of zoonotic and anthropozoonotic transmission between animals and their human caretakers. Animals in research colonies may have varied origins and travel histories, allowing for the introduction of bacterial strains from multiple sources. The current study evaluates MRSA carriage in a population of laboratory macaques, showing prevalence rates lower than those observed in previous studies of NHP but close to that of the hospitalized human population, with increased rates of carriage associated with greater degrees of veterinary intervention. However, the isolates in the studied population do not belong to prevalent local (Chicago, IL) human sequence types, with some seen sporadically in human populations in Asia and Australia, some currently undescribed in humans, and others that were identified during the course of this work. Our data suggest these are NHP-adapted MRSA strains that can be harbored in laboratory colonies of NHP.

Materials and Methods

Animals. Samples obtained from 148 rhesus macaques (*Macaca mulatta*) and 152 cynomolgus macaques (*Macaca fascicularis*) were used in a cross-sectional analysis. The study population comprised all macaques present in the institution's NHP colony at the initiation of the study, as well as all newly arrived animals until a total study population of 300 animals was achieved. All animals were housed in accordance with the *Guide for the Care and Use of Laboratory Animals*,[12] Public Health Service Policy,[24] and the Animal Welfare Act[1] and Regulations[2] in an AAALAC-accredited institution. All animal procedures described were determined, in consultation with the institution's IACUC, to be part of standard veterinary care and that a separate IACUC approval was unnecessary. Animals were maintained in an indoor facility kept at 22 ± 2 °C, 30% to 70% humidity, with 100% conditioned air and 12 to 15 air-changes hourly and lighting on a 12:12-h light:dark cycle (lights on, 0600). NHP were kept in visual and auditory contact with congenerics and cohoused in pairs whenever possible. Animals had access to toys and manipulanda in the cage, and speakers in the room provided auditory enrichment through music and natural sounds. Animals were fed 15% Monkey Diet (8714, Harlan-Teklad, Madison, WI) in weight-appropriate amounts once daily and had free access to municipal tap water. Foraging material or fresh produce was offered once daily. All NHP tested negative for STLV and SIV by serology and negative for SRV types 1 through 5 by serology and PCR analysis. All animals were determined to be free of tuberculosis through twice-yearly skin testing and were deemed healthy by annual physical examination, including evaluation of hematology, serum chemistry, and fecal flotation.

Sample collection. Swabs were taken from the nares while NHP were sedated under ketamine (10 mg/kg IM; Henry Schein, Dublin, OH) or ketamine (10 mg/kg IM)–xylazine (1 to 2 mg/kg IM; Lloyd Laboratories, Shenandoah, IA) for routine healthcare or study-related purposes. Nasal cultures were collected over a period of 9 mo, with a single culture swab collected from each animal. A swab (Liquid Stuart's Minitip Swab, Becton–Dickinson, Sparks, MD) was inserted into one nare until resistance was met or to 2 cm. The swab was then slowly rotated against the nasal mucosa while being retracted from the first nare; this process was repeated by using the same swab in the second nare. The swab was immediately placed in the sterile collection tube, stored at 4 °C, and transported to the University of Illinois Veterinary Diagnostic Lab within 24 to 48 h.

Isolation of methicillin-resistant and -sensitive *S. aureus* strains and identification of carriers. Swabs were immersed in 1 mL trypticase soy broth to remove and suspend bacteria from the nasal secretions. A fresh swab was used to transfer the suspension and inoculate plates of Columbia blood agar, Columbia blood agar containing colistin and naladixic acid, mannitol salt agar, and oxacillin (6 µg/mL) in agar (Remel Microbiology Labs, ThermoFisher Scientific, Lenexa, KS). After overnight incubation, staphylococcal isolates (a minimum of 3 colonies per plate for 4 media) demonstrating hemolysis or acid production from mannitol were subcultured to oxacillin-containing medium. Another 3 colonies were selected from the oxacillin medium and replated on Columbia blood agar to confirm their identification as staphylococci. Catalase and coagulase tests were performed to screen putative *S. aureus* isolates. Trek Sensititire GPID (ThermoFisher Scientific) or GP2 panels (Biolog, Hayward, CA) were used to confirm the identity of any atypical isolates. Representative isolates identified as *S. aureus* that grew on agar containing 6 µg/mL oxacillin were characterized regarding the minimal inhibitory concentration of oxacillin by using E-strips (bioMérieux, Lombard, IL). Oxacillin-resistant (≥4.0 µg/mL) isolates were confirmed phenotypically as *S. aureus* by means of MALDI–TOF mass spectrometry using the Bruker Biotyper (Bruker Daltonics, Billerica, MA) according to the manufacturer's tube extraction methods. A Main Spectrum library was created for further comparison of the isolates.

Isolates were genetically confirmed as MRSA through *mecA* PCR testing. A single colony of *S. aureus* was inoculated into 1 mL trypticase soy broth and incubated overnight. Broth cultures were centrifuged for 10 min at 6000 × *g* to pellet the cells. The cell pellets were resuspended in 180 µl of lysostaphin (200 µg/mL; 20 mM Tris-HCl [pH 8.0], 2 mM EDTA, 1.2% Triton; Sigma-Aldrich, St Louis, MO) and incubated at 37 °C for 30 min. After the addition of 20 µL proteinase K (stock, 20 mg/mL) and 200 µL of buffer AL, the tubes were incubated at 55 °C for 30 min followed by 10 min at 95 °C. Genomic DNA was extracted from these lysates by using QiaAmp DNA Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA was eluted in 100 µL of buffer AE. All buffer components used were

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part of the QiAmp – BioSprint 96 One-for-All Vet Kit (Qiagen, Leipzig, Germany). PCR analysis for *mecA* was performed as previously described.[30] Isolates were identified as MRSA when *S. aureus* was isolated and the *mecA* gene was present. Isolates identified as *S. aureus* that lacked *mecA* were classified as MSSA. In addition, the subset of randomly selected MSSA isolates used for antimicrobial susceptibility testing was confirmed to lack the *mecA* gene. Other staphylococci were classified on the basis of coagulase testing and hemolysis pattern on blood agar. NHP were classified as MRSA carriers when at least one MRSA isolate was obtained on nasal culture; MSSA carriers were identified in light of isolation of at least one *S. aureus* isolate on nasal culture but without MRSA isolates. Animals were determined to be noncarriers only when no *S. aureus* organisms were identified.

Antimicrobial sensitivity. Antimicrobial sensitivity was performed by using GPALL 3F (Sensititre, West Lake, OH) and enrofloxacin E-strips (bioMérieux, Marcy-l'Étoile, France) for all MRSA carriers (n = 19) and a subset of MSSA carriers (n = 15). MSSA carriers for analysis were selected by using the random-number generator function in Excel (Microsoft, Redmond, WA). Interpretation was based on Clinical and Laboratory Standards Institute criteria for companion animals (category A of document Vet A-04 S-1) or humans (category B of document M-100 S-23) for included antibiotics.[4] Drugs included in the analysis were selected on the basis of their usage in veterinary medicine, the presence of interpretative criteria, and the ability to obtain representative drugs from commonly used classes of antibiotics. Antibiotic-specific minimal inhibitory concentrations were determined by using TREK Sensititre Panel GPALL (ThermoFisher Scientific) for ampicillin, ceftriaxone, chloramphenicol, clindamycin, erythromycin, gentamicin, oxacillin, penicillin, rifampin, tetracycline, and trimethoprim–sulfamethoxazole (TMS). Minimal inhibitory concentrations of enrofloxacin and vancomycin were determined by using E-strips (bioMérieux).

***blaZ* PCR analysis.** MRSA isolates and the majority of MSSA isolates that were evaluated for antimicrobial susceptibility underwent PCR for the *blaZ* gene, which encodes penicillin resistance. PCR analysis was performed as described previously with slight modification.[10] The PCR mixture consisted of 32 pmol of each primer (stau-bla_z-fwd, 5′ CAA AGA TGA TAT AGT TGC TTA TTC TCC 3′; stau-bla_z-rev, 5′ TGC TTG ACC ACT TTT ATC AGC 3′), 100 ng of genomic DNA, and an Illustra PuReTaq Ready-To-GoPCR Analysis Bead (GE Healthcare Life Sciences, Pittsburg, PA) in a total volume of 25 µL. The following thermal cycling conditions were applied: initial denaturation at 95 °C for 5 min; 35 amplification cycles each consisting of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 30 s; and a final extension at 72 °C for 7 min. Amplification of a 421-bp product confirmed the presence of the bla_Z β-lactamase gene and penicillin resistance. PCR products were electrophoresed at 150 V for 50 min on a 2% agarose gel containing 0.5 µg/mL ethidium bromide.

Acquisition of demographic information. Complete medical records from time of colony entry and, when appropriate, importation into the United States (USFW 3-177), were obtained for all study participants (n = 300), and information on species, sex, age, weight, length of time in colony, country of origin, length of time in the United States, investigator, IACUC protocol assignment, history of surgical and dental procedures, history of illness or injury, history of antibiotic treatment, and history of steroid treatment was extracted (Table 1).

Statistical analysis of demographic information. The association between the demographic factors with the response subgroups (MRSA carrier status) was examined. For categorical factors (for example, sex, species), the χ^2 test and the Fisher exact test were used as appropriate. The Fisher exact test was applied when there were 5 observations or fewer in one or more subcategories. For continuous factors (for example, age, body weight), a logistic regression model was used for the binary response variable (MRSA carrier and noncarrier). The analyses were implemented by using R 3.2.2.[25] A P value of less than 0.05 was considered significant.

Molecular typing. One or more isolates from 18 of 19 MRSA-positive animals underwent identity confirmation by using the Staphaurex Plus Test (Remel) followed by molecular typing involving SCCmec typing, multilocus sequence typing (MLST), and PCR amplification for the detection of the *arcA* gene within the arginine catabolic mobile element (ACME *arcA*) and Panton–Valentine leucocidin (PVL) as described.[7]

MLST was performed by DNA sequencing of the PCR amplicons of 7 *S. aureus*-specific housekeeping genes by using primer sets described on the *S. aureus* organism page of the MLST Database.[16] Sequence types were assigned through submission of sequences to the MLST web portal, which compares the alleles deposited on the *S. aureus* organism page.

Results

Carriage rate. In the 300 macaques studied, *S. aureus* was isolated from the nares of 58.7% (n = 176), representing 50.7% (n = 75) of the rhesus macaques and 66.4% (n = 101) of the cynomolgus macaques. Of the total study population, 6.3% (n = 19; 8 rhesus, 11 cynomolgus) yielded *S. aureus* that supported amplification of the *mecA* gene and were classified as MRSA carriers (Figure 1). The remaining *S. aureus* carriers, accounting for 52.3% (n = 157) of the total population, did not have phenotypic resistance to methicillin or support amplification of the *mecA* gene and were classified as MSSA carriers. Of the total 176 animals from which *S. aureus* was isolated, 10.8% were classified as MRSA carriers, with the remaining 89.2% classified as MSSA carriers.

Demographic analysis. Statistical analysis of information from medical records revealed no significant association between MRSA carrier status and species, sex, age, body weight, length of time since entry into the University of Illinois at Chicago primate colony, country of origin, length of time in the US, assigned investigator, IACUC protocol assignment, presence of an implanted device, or history of illness or injury.

A history of at least one surgical procedure was significantly ($P = 2.45 \times 10^{-3}$) linked to MRSA carriage. Macaques that underwent surgical procedures had histories documenting between 1 and 4 surgical procedures, but there was no trend toward increased rates of carriage according to the number of surgical procedures. A history of at least one dental procedure was significantly ($P = 2.91 \times 10^{-3}$) linked to MRSA carriage. Dental procedures included endodontic procedures and surgical extractions. Macaques that underwent dental procedures had histories documenting between 1 and 6 dental procedures, but there was no trend toward increased rates of MRSA carriage according to the number of dental procedures. A history of antibiotic treatment was significantly ($P = 4.32 \times 10^{-3}$) linked to MRSA carriage rate; 34.7% (n = 104) of the study population had a documented history of receiving at least one antibiotic during their residence in the colony. The majority of these animals (n = 92) received cefazolin, 40 received clindamycin, and few (less than 10 each) had histories that included administration of ampicillin–sulbactam, amoxicillin–clavulanic acid, ceftiofur, enrofloxacin, erythromycin, or TMS. Within the population of animals that had a history of antibiotic administration, no single

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Table 1. Significance of demographic factors relative to MRSA carrier status

General	Factor	Category	MRSA+	MRSA–	Analysis method	*P*
Animal	Species	*M. fasicularis*	11	137		
		M. mulatta	8	144	χ^2	0.590
	Sex	Male	14	188		
		Female	5	90	χ^2	0.769
	Age	<4 y	4	85		
		5–15 y	12	172		
		>15 y	3	24	Fisher exact test	0.442
	Weight	<5 kg	9	152		
		5–10 kg	9	100		
		>10 kg	1	24	Fisher exact test	0.323
Geographic history	Time in US	<1 y	4	91		
		1–5 y	10	75		
		>5 y	3	39	Fisher exact test	0.168
	Time in colony	0–42 d	3	72		
		43 d–1 y	7	112		
		1–3 y	7	64		
		>3 y	2	33	Fisher exact test	0.559
	Vendor	16 categories	na	na	Fisher exact test	0.599
	Origin type	Academic	0	5		
		Import	17	218		
		National Primate Center	2	31		
		Pharmaceutical	0	20		
		Unknown	0	3	Fisher exact test	0.795
Research use	Protocol	24 protocols	na	na	Logistic regression	None significant
	Investigator	A	0	16		
		B	1	36		
		C	0	30		
		D	11	78		
		E	5	74		
		F	2	47	Fisher exact test	0.149
Veterinary intervention	**History of surgery**	**Yes**	**9**	**46**		
		No	**10**	**231**	**Fisher exact test**	**0.003**
	No. of surgeries	1	7	27		
		2	2	5		
		3	0	10		
		4	0	1	Fisher exact test	0.534
	History of dental procedure	**Yes**	**8**	**38**		
		No	**11**	**239**	**Fisher exact test**	$\mathbf{2.91 \times 10^{-3}}$

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Table 1. Continued

General	Factor	Category	MRSA+	MRSA–	Analysis method	P
	No. of dental procedures	1	7	26		
		2	1	8		
		3	0	2		
		4	0	1		
		6	0	1	Fisher exact test	0.847
	History of clinical incident	Yes	9	69		
		No	10	207	Fisher exact test	0.055
	Implant	Yes	1	23		
		No	18	242	Fisher exact test	1
	History of steroid administration	**Yes**	**5**	**9**		
		No	**14**	**272**	**Fisher exact test**	$\mathbf{0.824 \times 10^{-3}}$
	History of antibiotic administration	**Yes**	**13**	**91**		
		No	**6**	**183**	**Fisher exact test**	$\mathbf{4.32 \times 10^{-3}}$
	Specific antibiotic administered	7 categories	na	na	Fisher exact test	None significant

na, not analyzed
P values less than 0.05 are considered significant. Significant factors are bolded.

drug was significantly linked to MRSA carriage rate. A history of steroid treatment had a statistically significant link to MRSA carriage, with the smallest P value ($P = 8.24 \times 10^{-4}$). All animals in the study population with a history of steroid use received oral prednisone as part of a single experimental protocol (Table 1).

Molecular characterization of isolates. MRSA isolates had DNA extracted and were analyzed for the presence of virulence genes, multilocus sequence type, and SCCmec type (Table 2). All isolates were negative for PVL, which is commonly carried by community-associated (CA) MRSA strains in the United States. In addition, all isolates were negative for the ACME *arcA* gene, which is associated with the most common human CA-MRSA clone in the United States, USA300/ ST8. However, 94.4% of the macaque MRSA isolates were positive for *blaZ*, which codes for penicillin resistance.

According to MLST and SCCmec typing, 73.7% of MRSA carriers (n = 14) harbored ST188-SCCmec IV isolates; 15.8% (n = 3) of isolates, from 3 individuals, were identified as ST3268-SCCmec V; and a single MRSA isolate was identified as a novel sequence type, ST3478, carrying SCCmec V. No macaque had more than one MRSA sequence type isolated. However, on repeat analysis, one sample was determined to contain heterogeneous strain types, consisting of 1 MRSA isolate and 2 MSSA isolates, which also underwent MLST. In addition to a ST188-SCCmec IV MRSA, this sample included 2 previously undescribed MSSA isolates, ST3479 and ST3480. These isolates likely grew on oxacillin medium due to overproduction of β-lactamase, and 1 of the 2 MSSA was positive for *blaZ*. Alternately, the 2 MSSA could have been cocultured on the oxacillin-containing agar, where they benefited from the methacillinase produced by the MRSA strain.

Antibiotic susceptibility. Isolates underwent antibiotic susceptibility testing by using gram-positive sensitivity panels and enrofloxacin test strips to identify antibiotic resistance patterns for antibiotics in veterinary use. This analysis revealed remarkable differences between MSSA and MRSA isolates (Tables 3 and 4). The prevalence of antibiotic resistance among MSSA isolates tested was low: 87% of nontyped MSSA isolates were susceptible to enrofloxacin. However, 1 of the 2 novel strains of MSSA, ST3480, was resistant to enrofloxacin. All nontyped MSSA were susceptible to all other nonβ-lactam antibiotics tested, with the exception of a single MSSA isolate that was intermediately resistant to chloramphenicol. Compared with other MSSA strains, ST3479 and ST3480 had higher levels of resistance, with both showing resistance to clindamycin and erythromycin and intermediate resistance to vancomycin (Table 5). The high number of isolates exhibiting phenotypic susceptibility to penicillin among the MSSA isolates was surprising given the nearly universal presence of *blaZ*, which encodes the penicillin-hydrolyzing enzyme β-lactamase, among *S. aureus* isolates from human and animal populations.[22] Accordingly, 80% of MSSA isolates analyzed were *blaZ*-negative, confirming penicillin susceptibility.

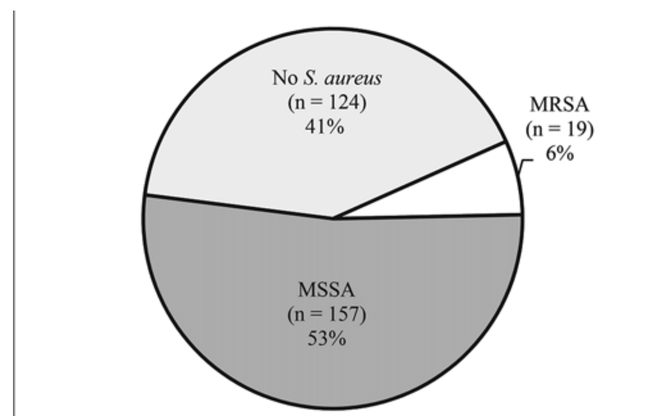

Figure 1. Carriage of MSSA and MRSA in relation to total study population (n = 300).

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Table 2. Presence or absence of *mecA*, *PVL*, ACME *arcA*, and *blaZ* genes grouped according to methicillin resistance and sequence type.

		mecA		*PVL*		ACME *arcA*		*blaZ*	
		+	−	+	−	+	−	+	−
MRSA	ST188	14	0	0	14	0	14	13	1
	ST3268	3	0	0	3	0	3	3	0
	ST3478	1	0	0	1	0	1	1	0
	not typed	1	0	Not done		Not done		1	0
MSSA	ST3479	0	1	0	1	Not done		1	0
	ST3480	0	1	0	1	Not done		0	1
	not typed	0	13	Not done		Not done		2	11

MRSA ST188 (n = 14), MRSA ST3268 (n = 3), MRSA ST3478 (n = 1), MRSA-not typed (n = 1), MSSA ST3479 (n = 1), MSSA ST3480 (n = 1), MSSA-not typed (n = 13).

Table 3. Percentage (%) of nontyped MSSA isolates (n = 15) susceptible to antimicrobials

	Susceptible	Intermediate	Resistant
Enrofloxacin	87	0	13
Ampicillin	100	0	0
Ceftriaxone	100	0	0
Chloramphenicol	93	7	0
Clindamycin	100	0	0
Erythromycin	100	0	0
Gentamicin	100	0	0
Oxacillin	100	0	0
Penicillin	100	0	0
Rifampin	100	0	0
Tetracycline	100	0	0
TMS	100	0	0
Vancomycin	100	0	0

As expected for MRSA, isolates were uniformly resistant to β-lactam antibiotics. A single MRSA isolate belonging to ST188 showed phenotypic susceptibility to penicillin and ampicillin, testing negative for the *blaZ* gene. The remaining MRSA isolates were *blaZ* positive (Table 2). MRSA isolates had a greater tendency to be resistant to nonβ-lactam antibiotics than the MSSA in our study population. Among the 9 nonβ-lactam drugs tested, all MRSA isolates were resistant to at least 1 drug, with the majority resistant to 3 or 4 additional drugs. However, none of the MRSA isolates in our study population was resistant to rifampin or vancomycin. No clear patterns regarding antibiotic resistance were present when isolates were stratified according to ST (Table 5).

Discussion

We characterized the prevalence of MRSA colonization in a representative academic research colony of NHP, with the goals of determining factors linked to increased rates of carriage and gaining insight into the origin of strains present within our study population. It is noteworthy that the MRSA carriage rate of 6.3% in our NHP is close to that reported in hospitalized human populations but much lower than carriage rates in human ICU and long-term care settings[33] and in other populations of laboratory macaques and chimpanzees.[11,31] This difference may reflect that 25% of the study population had been in the colony for less than 42 d, the minimal length of the quarantine period, during which no study-related activities were conducted, or the variable degree to which animals had undergone study-related or veterinary manipulation. However, we did find a surprisingly high rate of nasal *S. aureus* carriage, with the bacteria isolated from 58.7% of the study population; this rate is almost twice that in many studies of human CA-MRSA and previous data from laboratory macaques.[18,22,38] It is unknown whether this difference was due to culture methods that were more sensitive for detection of *S. aureus* or whether this high carriage rate is a true reflection of the carriage rate in the population studied.

Our analysis of risk factors showed a significant association between MRSA carriage and veterinary or experimental interventions that included dental procedures, surgical procedures, antibiotic administration, and steroid administration. Although the factors identified do appear to be linked to MRSA carriage, determining the individual effect of each factor is challenging. At our institution, perioperative antibiotics are regularly given, and antibiotics are frequently administered in conjunction with dental procedures where infection is a component of the presenting pathology. In addition, the protocol under which animals received oral prednisone included a surgical procedure, for which perioperative antibiotics were given. Furthermore, due to advanced age, many animals under this protocol had dental pathology, requiring dental procedures prior to experimental use. Although it is impossible to ascertain the influence of each individual factor within the current study population, the current data support increased veterinary intervention affecting MRSA carriage status. These risks correlate with many factors thought to predispose the hospitalized human population to MRSA infection. However, in the human literature, although antibiotic administration does predispose to MRSA carriage,[18] there is no

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Table 4. Antimicrobial susceptibility of MRSA isolates, grouped according to sequence type; data in each column are given as percentage of total isolates

	ST188 (total n = 14)			ST3268 (total n = 3)				
	S	I	R	S	I	R	ST3478 (n = 1)	Not typed (n = 1)
Enrofloxacin	0%	0%	100%	0%	0%	100%	S	S
Ampicillin	7%	0%	93%	0%	0%	100%	R	R
Ceftriaxone	0%	14%	86%	0%	33%	67%	I	R
Chloramphenicol	7%	93%	0%	33%	67%	0%	I	I
Clindamycin	14%	0%	86%	0%	67%	33%	S	R
Erythromycin	14%	0%	86%	0%	67%	33%	S	R
Gentamicin	7%	0%	93%	0%	33%	67%	R	R
Oxacillin	0%	0%	100%	0%	0%	100%	R	R
Penicillin	7%	0%	93%	0%	0%	100%	R	R
Rifampin	100%	0%	0%	100%	0%	0%	S	S
Tetracycline	86%	0%	14%	33%	0%	67%	R	S
TMS	43%	0%	57%	67%	0%	33%	S	R
Vancomycin	100%	0%	0%	100%	0%	0%	S	S

I, intermediate; R, resistant; S, susceptible

Table 5. Antimicrobial susceptibility of newly described MSSA isolates ST3479 and ST3480

	ST3479	ST3480
Enrofloxacin	Sensitive	Resistant
Ampicillin	Resistant	Sensitive
Ceftriaxone	Sensitive	Sensitive
Chloramphenicol	Sensitive	Sensitive
Clindamycin	Resistant	Resistant
Erythromycin	Resistant	Resistant
Gentamicin	Sensitive	Sensitive
Oxacillin	Sensitive	Sensitive
Penicillin	Resistant	Sensitive
Rifampin	Sensitive	Sensitive
Tetracycline	Sensitive	Sensitive
TMS	Sensitive	Sensitive
Vancomycin	Intermediate	Intermediate

evidence that the remaining factors are linked to increased risk of MRSA carriage compared with carriage of MSSA.

No associations between MRSA carriage rate and time in the colony, animal origin, or the protocol or investigator to which an animal was assigned were identified, suggesting that MRSA does not enter the colony from a single origin or transfer between animals that share human handlers or equipment. The lack of significant associations for these factors may be due to the fact that our study population was extremely heterogeneous and, with only a few positive animals, it is difficult to show significant statistical association. Although our methods grouped together animals that shared an experimental protocol or investigator, these signifiers did not distinguish between those animals that had undergone protocol-related manipulation and those awaiting use. This situation may explain the statistically significant link between veterinary or experimental intervention and carriage rate, whereas no association occurred between either investigator or protocol and carriage rate, despite the fact that many interventions linked to increased carriage rate were protocol-based.

Antibiotic susceptibility patterns between MRSA and MSSA isolates differed widely. MSSA in our study population had low levels of resistance to most antibiotics tested, including penicillin, thus suggesting that isolates in our population differ from those in the human population, where penicillin resistance is currently greater than 90% in human clinical isolates[22] and 78% in commensal nasal flora.[36] Penicillin resistance is also prevalent and well documented in groups of animals with extensive human contact or exposure to antibiotics, including companion animals, livestock, and NHP. A study in captive lion tamarins demonstrated penicillin resistance in all *S. aureus* isolates,[21] and studies of *S. aureus* carried by sanctuary chimpanzees showed as many as 75% of isolates to be resistant to penicillin.[28,29] In the chimpanzee study,[28,29] a high degree of concordance with sequence types found in the local human population was observed, supporting anthropozoonotic spread of penicillin-resistant isolates. In contrast, the high degree of antibiotic susceptibility among MSSA isolates in the current study indicates that these strains differ from those circulating in the human population.

MRSA isolates in our population showed very broad antibiotic-resistance patterns, which is more characteristic of health-care-associated MRSA than CA-MRSA. Although most isolates were resistant to 3 or 4 nonβ-lactam drugs, there were no consistent patterns of susceptibility when isolates were grouped according to sequence type. All of the MRSA isolates from our study population carried SCCmec type IV or V, which are present among CA-MRSA. These mobile genetic elements are

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smaller than other types and generally associated with more virulent strains. However, none of these isolates carried the *PVL* gene, which codes for a pore-forming toxin associated with increased virulence that is commonly identified in CA-MRSA isolates.[9]

Evidence supports the frequent zoonotic and anthropozoonotic transmission of methicillin-resistant staphylococcal species. For example, MRSA strains identified in pets frequently mirror those seen in the local human population,[40] and pork producers are frequently carriers of ST398, a strain common in pigs.[5,6] Our data, in contrast to observations in captive laboratory chimpanzees,[11] show a lack of local human strains and suggest the presence of NHP-associated MRSA strains.

ST188-SCCmec IV accounted for almost 75% of the MRSA isolates that we found in our study population. Notably, ST188-SCCmec-IVa was identified in nasal samples from NHP at a National Primate Center, as well as in the local environment and in a nasal sample from a member of the research staff.[31] This ST has been identified in the human population in the Asia–Pacific region, primarily as MSSA, but has not been documented in the local human population of Chicago, where the current study was conducted. ST188 is most closely related to MRSA of clonal complex 1, which contains USA400, a common CA-MRSA. However, genetic differences between the 2 groups are dispersed across the genome in a manner that suggests multiple recombination events over a complex natural history.[16] Reports of ST188 in humans vary, including association with both hospital and community-acquired infections and in both *PVL*-positive and -negative forms.[13,23,42] In addition, ST188 MRSA has been found in raw chicken in China.[39] The isolates of ST188 in NHP at the earlier-mentioned National Primate Center as both MRSA and MSSA were closely related to each other but were easily differentiated from the human isolate of ST188 from Hong Kong used to generate the draft genome sequence.[13,31] The ST188 isolates in our study showed variable phenotypic resistance to all antibiotics tested, except for oxacillin, to which all were resistant, and rifampin and vancomycin, to which all were susceptible. These findings suggest that despite a shared sequence type, these isolates comprise a heterogeneous population.

The second genotype identified, ST3268-SCCmec V, was present in 3 of the 19 MRSA carriers in our study population. These animals consisted of 2 rhesus and 1 cynomolgus macaque. The rhesus macaques were imported by 2 separate suppliers from China in 2013 and 2014, whereas the cynomolgus macaque was imported from Cambodia in 2008. All 3 NHP arrived in separate shipments within a 1-mo span of time and were sampled for carriage between 19 and 113 d after colony entry. ST3268 has not been reported in humans, but ST3268-SCCmec V was identified in macaques at the earlier-mentioned National Primate Center.[31] The most closely related strain is ST2817, a human clinical MRSA isolate identified in Singapore.[16] Isolates of ST3268 varied in resistance to all nonβ-lactam antibiotics tested, except for enrofloxacin, to which all were resistant, and rifampin and vancomycin, to which all were susceptible. The third sequence type identified in our colony was ST3478. This previously undescribed ST was identified from a single animal and, of the nonβ-lactam drugs tested, was resistant to gentamicin and tetracycline, with intermediate resistance to chloramphenicol. This novel MRSA background has multiple alleles (*glpF*, *pta*) that do not correspond to previously described sequences, but it is most closely related to ST2096, an MSSA isolated from a macaque nasal swab in the Netherlands that does not fit within previously described clonal complexes.[37] A previous study investigating *S. aureus* nasal carriage in rhesus macaques found that 59% of isolates were previously undescribed and unrelated to identified human strains, thus supporting NHP-specific MSSA lineages.[37] In our current study, the identification of isolates that are unidentified in humans yet that have been identified in unrelated populations of laboratory macaques is consistent with this previous finding and suggests the presence of NHP-adapted MRSA with the potential to pass between macaques and humans.

Our findings provide no evidence of MRSA entering the study population from the local human population in Chicago; however, the potential for zoonotic transmission bears further investigation. ST188, the most prevalent isolate type identified in the study population, is most frequently observed in Asia, where the majority of new arrivals to the colony originated. This strain may enter from the human population in Asia or may be an NHP-specific strain transmitting into humans. Further investigation of MRSA carriage in both humans and animals associated with macaque breeding facilities and analysis of macaque populations from multiple origins may shed light on this dynamic.

Our current study describes the nasal carriage of MRSA in a population of laboratory macaques. The carriage rate in the study population was comparable to that of many studied hospitalized human populations. But, much as variation exists between human populations, the carriage rate may differ widely between facilities, with our results dependent on characteristics specific to the population studied. However, the current findings place laboratory NHP in the context of broader patterns of emergence of antimicrobial resistant bacteria in humans and animals. Taken as a whole, our data strongly support the potential for NHP-associated MRSA strains and lay the groundwork for further investigations of MRSA in NHP.

Acknowledgments

We thank Alex Castenada and Heather Charles (UIC) for assistance with sample collection and processing, Kat Coda (UIC) for assistance collecting demographic information, and Dr Saraswathi Lanka (UIUC-VDL) for *mec*A and *bla*Z PCR analysis. This article was prepared while Susan (Daum) Boyle-Vavra was employed at the University of Chicago. The opinions expressed in this article are the authors' own and do not reflect the view of the National Institutes of Health, the Department of Health and Human Services, or the United States Government.

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