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Original Research

Bacterial Genotype, Carrier Risk Factors, and an Antimicrobial Stewardship Approach Relevant to Methicillin-resistant *Staphylococcus aureus* Prevalence in a Population of Macaques Housed in a Research Facility

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Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a significant problem for human and animal health and can negatively affect the health status of macaques and other nonhuman primates (NHP) in research colonies. However, few publications provide guidance on the prevalence, genotype, or risk factors for macaques with MRSA and even fewer on how to effectively respond to MRSA once identified in a population. After having a clinical case of MRSA in a rhesus macaque, we sought to determine the MRSA carrier prevalence, risk factors, and genotypes of MRSA in a population of research NHPs. Over a 6-wk period in 2015, we collected nasal swabs from 298 NHPs. MRSA was isolated from 28% (n = 83). We then reviewed each macaque's medical record for a variety of variables including animal housing room, sex, age, number of antibiotic courses, number of surgical interventions, and SIV status. Analysis of these data suggests that MRSA carriage is associated with the room location, age of the animal, SIV status, and the number of antibiotic courses. We used multilocus sequence typing and *spa* typing on a subset of MRSA and MSSA isolates to determine whether the MRSA present in NHPs was comparable with common human strains. Two MRSA sequence types were predominant: ST188 and a novel MRSA genotype, neither of which is a common human isolate in the United States. We subsequently implemented antimicrobial stewardship practices (significantly reducing antimicrobial use) and then resampled the colony in 2018 and found that MRSA carriage had fallen to 9% (26/285). These data suggest that, as in humans, macaques may have a high carrier status of MRSA despite low clinically apparent disease. Implementing strategic antimicrobial stewardship practices resulted in a marked reduction in MRSA carriage in the NHP colony, highlighting the importance of limiting antimicrobial use when possible.

Abbreviations and Acronyms: MLST, multilocus sequence typing; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*

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Introduction

The global crisis of bacterial antibiotic resistance is exemplified by methicillin-resistant *Staphylococcus aureus* (MRSA) colonization of humans and animals. Since resistance to penicillin was first identified in the 1940s, *S. aureus* has shown a remarkable ability to develop resistance to newly introduced antibiotics.[6,62] During this time, MRSA colonization rates have steadily grown in humans,[41,64] as have the number of hospitalizations attributed to MRSA.[40,54] In the USA the estimated prevalence of MRSA in the general population is approximately 1% to 2%.[75] Over 80,000 serious MRSA infections occurred in 2011 alone.[21] The US healthcare industry has some evidence of a decline in MRSA infections, but this decline somewhat slowed after 2012, and the number of affected patients is still high.[12,35] The estimated number of MRSA cases in hospitalized US patients was over 300,000 in 2017.[12]

While nasal colonization increases the risk of developing MRSA-associated disease, only a minority of people who carry MRSA will develop disease.[35] Furthermore, MRSA colonization rates are affected by multiple factors and are considerably higher in some settings, including among healthcare workers, who have a prevalence of approximately 5%.[24] Other individuals in high interpersonal contact settings such as college athletes and military recruits also have relatively high incidences of disease, and outbreaks have been reported in these populations.[75] In addition, other known risk factors for MRSA colonization

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include use of antibiotics[86] and a variety of factors associated with decreased immune status such as HIV/AIDS,[14,101] breaks in protective skin barriers, hygiene challenges,[63,70] indwelling medical device,[66] older age,[66] and crowded living conditions.[45]

Large-scale epidemiologic studies have been undertaken to better understand MRSA carriage rates and resultant infections in humans.[46] MRSA colonizes the human skin, gastrointestinal tract, and nasal passages and can cause clinically significant infections in subsets of colonized individuals.[9,42,43,48,92] The epidemiologic analysis of MRSA within populations has resulted in the broad categorization of MRSA into hospital-, community-, and livestock-acquired MRSA.[20,48,61,62] Genetic typing is used to determine relationships between different MRSA isolates and virulence factors.[60]

While substantial research has focused on the impact of MRSA on human health, relatively little published information about MRSA is available with regard to nonhuman primates (NHPs), whether wild, in zoos, or in captive research populations. Therefore, establishing a broader understanding of MRSA colonization and epidemiology is warranted for NHPs. However, MRSA colonization or infections in NHPs used in research has only recently been described and overall the literature is sparse.[1,26-28,44,65,73,77-79,85] A recent systematic review/meta-analysis of the literature found only 7 studies focused on wild NHP, among other wild animal studies.[1] In some of the wild primate studies, the prevalence of MRSA was low, ranging from 0% to 5.3% depending on location,[77,79] however, the prevalence of drug resistance among *S. aureus* isolates may increase when the NHPs are in close proximity to humans[78] or in areas in which direct and indirect exposure to humans is common.[28]

As in wild populations, the overall literature from captive populations of NHPs is limited. Prior reports of MRSA prevalence in captive NHPs range from 6% to 22% in macaques[26,44,51,85,87] and up to 69% in chimpanzees;[27] however, some of these reports may represent uncommonly high rates of carriage due to a bias in performing prevalence studies after recognition of MRSA infections in the population. A study of MRSA in captive chimpanzees identified MRSA genotypes that appeared to be human in origin (community acquired type ST300),[27] and other studies in macaques have identified *S. aureus* (both MRSA and MSSA) that are either rare human genotypes or unique to macaques.[26,51,73,85,90] Interest is also emerging for the use of NHPs to study human *S. aureus* colonization,[17,83,90] but this research does not appear to specifically focus on MRSA. Finally, several reports have been based on small macaque groups or individual animals. For example, in one report, a high percentage of a small number of macaques cultured positive for MSSA and MRSA.[65] Other reports describe a small number of cases, for example in catheter tracts or cranial implants.[15,49,88] These reports are important for veterinarians managing colonies in which these procedures are commonly performed, but they probably provide little overall information regarding risk factors and management practices that could reduce MRSA carriage in larger research colonies. While some facilities have focused on eradication after identification of MRSA, an equivalent or even more important focus is prevention. To that end, facilities should develop antimicrobial stewardship practices[5] to improve NHP health and reduce the risk for MRSA carriage. While the CDC defines antimicrobial stewardship as a way to reduce antibiotic resistance, some facilities may not yet view stewardship as a pressing issue.[13]

Many questions remain unanswered with regard to MRSA in captive NHP including the risk factors associated with colonization, how to reduce the prevalence of MRSA once it is identified in a population of NHP, and a better knowledge about the MRSA genotypes associated with colonization of research populations of NHP. To address these issues, we investigated the prevalence of and risk factors for MRSA carriage in Asian macaques in a research colony. We systemically surveyed groups of captive rhesus macaques (*Macaca mulatta*) and pigtailed macaques (*Macaca nemestrina*) to establish prevalence, MRSA genotypes and NHP risk factors that contribute to MRSA carriage, and subsequently we applied strategic antimicrobial stewardship practices[4,5] to the population to effectively reduce MRSA carriage.

Methods

Animal housing. All NHPs were housed at the National Institutes of Health and maintained in accordance with the *Guide for the Care and Use of Laboratory Animals*.[32] The facility is accredited by AAALAC International, and all animal use was humanely conducted and approved by the National Cancer Institute's Animal Care and Use Committee (NCI-ACUC). NHPs were screened initially during preventive healthcare examinations as part of the veterinary care program to determine the overall MRSA risk to the colony and staff after we identified a clinical case in one NHP. Follow-up testing was then included in a research protocol approved by the NCI-ACUC. Findings were discussed with the NCI-ACUC throughout and were used to guide veterinary oversight of antibiotic usage and stewardship in the facility.

The study was conducted in 3 different buildings (A, B, and C) with study animals in 22 holding rooms (A through V; Figure 1). Animals were housed in stainless-steel NHP caging either socially or individually, depending on ACUC-approved protocol requirements or on veterinary concerns, as based on the Animal Welfare Act and *The Guide for the Care and Use of Laboratory Animals*.[32]

Animals. The 2015 study population included rhesus (*Macaca mulatta*, $n = 291$) and pigtailed (*Macaca nemestrina*, $n = 7$) macaques. The macaques ranged in age from 1.9 to 19 y with both males ($n = 167$) and females ($n = 131$) in the study population. The 2018 study population included rhesus ($n = 271$) and pigtailed ($n = 14$) macaques. Thirty-eight animals were present in the colony at both time points. All macaques included were actively in other ongoing National Cancer Institute (NCI)-ACUC–approved research protocols and all surgery and other procedures were either conducted under approved research protocols or were required for clinical intervention and veterinary treatment.

Colony surveillance and record review. Over a period of 6 wk in early 2015, all animals ($n = 298$ individual macaques) were sedated with ketamine (10 to 20 mg/kg IM, Zetamine, VetOne, Boise, ID) for routine veterinary examinations as part of the preventive healthcare program. During these exams, sterile cotton tipped swabs (BactiSwab, Remel Lenexa, KS) were gently inserted into the nares, removed, and placed into Amies transport media. One swab was used for both the left and right nares. Several of the male monkeys also had samples collected from the prepuce. Swabs were processed on the day of collection, as described below. Nares swabs were similarly collected in 2018 over a period of approximately 5 months, when macaques ($n = 285$) were sedated with ketamine for other planned procedures, including routine veterinary examinations.

The 2015 health records from all macaques were reviewed, and the following data were collected: age, room location, sex, number of courses of antibiotics since arriving at the facility,

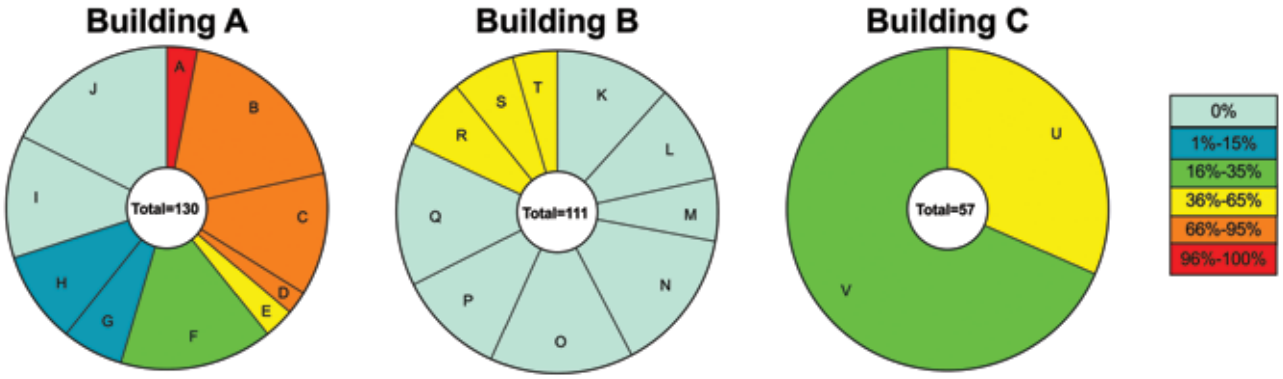

Figure 1. MRSA prevalence for animals in Building A (rooms A through J), Building B (rooms K through T), and Building C (rooms U through V). Size of each wedge represents the relative proportion of animals per room for the specified building. MRSA prevalence is indicated by the color of the wedge as described in the figure key. MRSA prevalence per building: Bldg A, 35%; Bldg B, 10%; Bldg C, 46%.

number of surgeries, number of courses of cephalosporins/enrofloxacin/other antibiotics, SIV status, length of time in the facility, and vendor of origin. A similar but refined data set was collected in 2018 and included courses of antimicrobials since arriving in the facility, number of surgeries, and age. Macaques arrived from a variety of other institutions and locations; this information was not used in the final analysis. A course of antibiotics was defined as any antibiotic prescription, whether for experimental or clinical reasons. In 2015 most macaques had undergone a multimodal cleanup regimen[7] according to facility practices implemented before the employment of this paper's authors; however, the data were collected and analyzed as total courses of antibiotic exposure and were not specific to the cleanup regimen. Surgery was defined as any major or minor operative procedure.

Antimicrobial stewardship practices were implemented in the facility between 2015 and 2018 and included several different approaches but generally followed recently published guidelines.[5] The cleanup regimen was ended as a standard facility practice; investigators who wanted to continue the clean-up regimen on their animals included this practice in their ACUC protocol. Enhanced diagnostic testing occurred at NHP vendors, in the quarantine facility, and in research facilities to improve detection of animals carrying pathogens of concern. The use of a course of cephalexin as a prophylactic was reduced during and after minimally invasive clean surgical procedures. Antibiotic use to treat diarrhea was reduced. Finally, antibiotics were only used when necessary and choice was driven, when possible, by culture and sensitivity.

MRSA culture and identification. Swabs were inoculated into thioglycollate broth, incubated overnight at 37 °C, and plated onto 3 separate plates: blood agar, HardyCHROM MRSA agar (Hardy Diagnostics, Santa Maria, CA), and phenylether alcohol (PEA) agar. Blood agar was used to screen for *S. aureus*. Any colonies morphologically consistent with betahemolytic staphylococci were subcultured and incubated for an additional 24 h at 37 °C. If colonies were morphologically consistent with *Staphylococcus*, catalase and coagulase tests were performed. If the colony was both catalase and coagulase positive, the Vitek-2 ID system was used to determine if the bacteria was *Staphylococcus aureus*. If identified as such, cefoxitin susceptibility was assessed by disk diffusion as per Clinical and Laboratory Standards Institute (CLSI) guidelines.[10] Cefoxitin-resistant isolates were presumptively identified as MRSA. The HardyCHROM MRSA agar was also used for screening. This agar is selective for MRSA, which grows as pink or magenta colonies. Colonies isolated on the selective agar were tested with both the Vitek-2 system and cefoxitin disks. PEA inhibits the growth of gram-negative colonies and was used to select for gram-positive bacteria. Again, colonies isolated on this agar were analyzed with the Vitek-2 system and, depending on the results, were tested for cefoxitin resistance. Some, but not all, MRSA colonies were further assessed using the disk diffusion Kirby-Bauer test with a broader set of antimicrobials (clindamycin, trimethoprim sulfonamide, vancomycin, cephalothin, ampicillin, ceftriaxone, erythromycin, kanamycin, penicillin G, tetracycline, cefepime, ceftiofur, gentamicin, and oxacillin).

Bacterial Genotyping. Multilocus sequence typing (MLST) and *spa* typing were performed on a subset of cultures collected from 29 macaques cultured in 2015 (23 MRSA and 6 MSSA; details presented below).[25,76] All genotyped isolates were from different macaques. One MSSA sample did not amplify for either MLST or *spa* typing and was excluded from the rest of the genetic analysis. Although testing every sample may have been beneficial, as would have whole genome sequencing, this effort was beyond the scope of this project and was not attempted. Instead, 29 samples were chosen from the original 298 samples that had been collected; these samples represented a variety of rooms (rooms B, C, F, J, L, R, S, U, and V), buildings (buildings A [n = 13], B [n = 10] and C [n = 6]), and experimental conditions. Because samples were selected to represent specific rooms across the whole program, the sampling was not completely random.

For MLST, *Staphylococcus aureus* strains were cultured at 37 °C overnight in tryptic soy broth with shaking. After cells were harvested by centrifugation, Staphylococcal genomic DNA was isolated and gene loci for MLST were PCR amplified according to previously published methods.[76] The *Staphylococcus aureus* MLST primers used for PCR amplification of gene loci have been described previously.[25] Purified PCR products were sequenced with the *Staphylococcus aureus* MLST primers using standard methods. Allele numbers and subsequent sequence types were determined by querying PCR product sequences against the allele sequences of the *Staphylococcus aureus* MLST database (http://saureus.mlst.net/). *spa* typing was performed as previously described[81] with types characterized using the Ridom *spa* server (http://SpaServer.ridom.de).

Statistical analysis. Data were analyzed within the R statistical language and environment using logistic regression, loglinear modeling, and categorical data analysis.[2,29] Univariate and multivariate analysis were performed and included

data from 288 macaques in the original 2015 cohort. The criterion variable of interest was MRSA status, a dichotomous outcome expressed as positive (1) or negative (0). Because both nasal and preputial swabs were obtained from a few macaques, a positive result on either was recorded as positive. However, the vast majority of the data represents results from nasal cultures. Continuous and categorical explanatory variables were analyzed with both univariate and multivariate logistic regression models to determine their effect on MRSA. To facilitate interpretation, some continuous variables (for example, age) were redefined as categorical variables (that is, 'young' [< 4 y, n = 87], 'mid' [4 to 5 y, n = 92], and 'old' [> 5 y, n = 109]). The Likelihood Ratio (LR) χ^2 test statistic was used in all logistic regression and loglinear models. Probability values less than 0.05 ($P < 0.05$) were considered significant.[89] Comparisons between 2015 and 2018 data were analyzed using GraphPad Prism v9.3.1.1 (GraphPad Software, La Jolla, CA). The nonparametric Mann–Whitney test was used to determine P values when comparing 2 groups (expressed as a mean ± 1 SD) that were not normally distributed and the χ^2 test was used to analyze contingency tables.

Results

Colony information and colony surveillance culture results from the 2015 cohort. Samples for MRSA culture were collected from the nares of 298 macaques in 22 animal housing rooms located in 3 buildings (A, B, or C; Figure 1). A smaller group of macaques (n = 75) also had samples collected from the prepuce. In these animals, some nares were positive when the prepuce was not. These animals were included in the analysis as MRSA carriers for analysis. The sampled macaques included both males (167) and females (131). Their ages varied, with an average of about 5.5 years. At the time of analysis, 49 animals had not had surgery while the remaining 249 had at least one surgery. The average number of surgeries was 2 per animal when including every animal and 4 per animal when excluding those with no surgical history. In total, 66 animals were known to be infected with SIV while 232 were not. Almost all animals had received antibiotics previously with an average of 5.2 antibiotic courses across all animals. The average number of courses of cephalexin, enrofloxacin, and other antibiotics was 1.9, 1.7, and 1.6 per macaque, respectively.

A total of 99 (33%) macaques cultured positive for *Staphylococcus aureus*; of these, 83 (28%) cultured positive for MRSA using the methods described above, while the other 16 (5%) were positive for MSSA. None of the 7 pig-tailed macaques (0 of 7) cultured positive for MRSA; one (1 of 7) cultured positive for MSSA. Positive culture results were not evenly distributed between rooms ($P < 0.001$); some rooms had a higher prevalence of MRSA carriage than did others (Figure 1). The prevalence of MRSA colonized macaques for buildings A and C were 35% and 46%, respectively, whereas it was only 10% in building B. Animals were not housed in different rooms based on sex, age, procedures, or study status, but rather an effort was made to house animals on the same or similar studies together. All areas had similar husbandry practices. Despite these similarities in research and husbandry practices, the rooms and buildings were strikingly different in prevalence of MRSA.

Sensitivity results could not be obtained for all samples and all antibiotics. However, the disk diffusion method was used to test for antimicrobial sensitivity in some animals. In general, MRSA isolates were sensitive to clindamycin, trimethoprim sulfonamide, vancomycin, and cephalothin but resistant to ampicillin, ceftriaxone, erythromycin, kanamycin, penicillin G, tetracycline, cefepime, ceftiofur, gentamicin, and oxacillin.

Genotyping results from the 2015 cohort. Multilocus sequence typing (MLST) was performed on 6 MSSA (samples 1 to 6) and 23 MRSA (Samples 7 to 29) isolates (Table 1). All 6 MSSA samples were identified as novel sequence types by MLST. MRSA samples were broadly distributed into 2 different groups by MLST; 8 of 23 were sequence type (ST) 188, which is found in humans, and 15 of 23 were one of either 2 novel MLST sequences (Novel A or Novel B; Table 1). Novel A and B MLST were the same for both MRSA and MSSA samples. They were identified here as novel because they had not previously been given ST numbers in the ST database.

Seven of 23 MRSA samples were identified as *spa* type *t189*; those isolates were also identified as ST188 on MLST. The remaining ST188 sample had a different *spa* type, t2174, that

Table 1. Genotyping results from MSSA (n = 6) and MRSA (n = 23) samples.

Sample	Bacterium	MLST	*aroC*	*aroE*	*glpF*	*gmk*	*pta*	*tpi*	*yqil*	*spa* Type	*spa* RiDOM Motif
1	MSSA	Novel A	1	14	N/F[a]	214	10	303	329	New 2	03-02-17-12-17-34-22-150
2	MSSA	Novel C	10	2	N/F[e]	2	10	303	329	*t13638*	210-23-02-34-17-34-34-17-23-34
3	MSSA	Novel B	14	253	1	2	N/F[c]	58	2	New 2	03-02-17-12-17-34-22-150
4	MSSA	Novel D	22	23	1	8	1	1	1	*t8397*	04-20-24-17-17-25
5	MSSA	Novel E	3	N/F[b]	1	105	6	1	10	New 3	04-20-25-16-23-24-17
6	MSSA	Novel F	3	N/F[b]	1	1	6	1	N/F[d]	New 3	04-20-25-16-23-24-17
7-13	MRSA	188	3	1	1	8	1	1	1	*t189*	07-23-12-21-17-34
14	MRSA	188	3	1	1	8	1	1	1	*t2174*	26-23-12-21-17-34
15	MRSA	Novel B	14	253	1	2	N/F[c]	58	2	*t13638*	210-23-02-34-17-34-34-17-23-34
16	MRSA	Novel A	1	14	N/F[a]	214	10	303	329	New 1	210-23-34-17-34-34-17-23-34
17-29	MRSA	Novel A	1	14	N/F[a]	214	10	303	329	*t13638*	210-23-02-34-17-34-34-17-23-34

MLST alleles: *aroC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, *yqil*
Novel A and B were the same between the MRSA and MSSA
[a]Closest in similarity to *glpF* allele 235
[b]Closest in similarity to *aroE* allele 89
[c]Closest in similarity to *pta* allele 4
[d]Closest in similarity to *yqil* allele 11
[e]Closest in similarity to *glpF* allele 129

differed from *t189* at a single repeat. Fourteen of 23 MRSA samples were identified as *spa* type *t13638*, with 13 of the 14 having the same novel sequence identified on MSLT (Novel A); the remaining isolate was another novel MSLT (Novel B; Table 1).

Univariate Analyses from the 2015 cohort. Of the 298 macaques from which cultures were collected in 2015, 10 could not be evaluated because of incomplete records, and thus they were not included in the univariate and multivariate analysis. The remaining 288 cultures were ultimately evaluated using only one isolate from each macaque. In the univariate analyses, age (AGE [continuous variable in years], $P < 0.0001$; AGE_categ (categorical variable with 3 levels: Young, Mid, Old, see methods for details), $P < 0.0001$), room (Room, $P < 0.0002$) and building (MS.bldg, $P < 0.0001$) were all significantly associated with a greater likelihood of MRSA carriage. Furthermore, the number of courses of antibiotics (nAB, $P < 0.0001$), the number of surgeries (nSx, $P < 0.0001$), the number of courses of cephalexin (nCep, $P < 0.0001$), simian immunodeficiency virus status (SIV, $P < 0.0001$), and length of time in the facility (FacTime, $P < 0.0001$) were also associated with MRSA carriage (Table 2). In 2015, common practice was to provide cephalexin as peri- and postoperative care for various surgeries and to administer courses of several antimicrobials as part of a clean-up protocol to all arriving animals.[7] Use of these practices had been markedly curtailed between the surveillance performed in 2015 and 2018. Sex (SEX, $P = 0.43$), was not a significant predictor of MRSA carriage, nor were the number of courses of enrofloxacin (nBay, $P = 0.44$) or other antibiotics (nOth, $P = 0.19$) (Table 2).

When age is analyzed as a continuous variable, an increase of one year in the age of a macaque multiplies the odds of MRSA carriage by exp (0.1670) = 1.18, or by 18% (Table 2). When age is analyzed as a categorical variable, it was defined as 1) Young, age less than 4 y; 2) Mid, age from 4 up to 5 y, and 3) Old, age over 5 y. In this categorical analysis, age was identified as a highly significant predictor of MRSA status ($P < 0.0001$). Mid-aged and young macaques did not have significant differences in the likelihood of carrying MRSA (OR = 2.4; ($P = 0.0761$). However, old macaques were about 7 times as likely (OR = 7.1) to carry MRSA than were mid-aged animals ($P < 0.0001$) and about 17 times more likely (OR = 17.2) to carry MRSA than were young ones ($P < 0.0001$).

The administration of a course of antibiotics increased the odds of MRSA carriage by 15%, ($P < 0.0001$). The experience of a surgery increased the odds of MRSA carriage by 23%, ($P < 0.0001$). One course of cephalexin therapy increased the odds of MRSA carriage by 38%, ($P < 0.0001$). SIV status increased the odds of MRSA by 146%. A year longer in the facility increased the odds of MRSA by 17% ($P < 0.0001$).

Multivariate Analyses from the 2015 cohort. To statistically adjust the estimated effects of each variable for differences in the distributions of the other independent variables, we performed multivariate analyses to determine the joint impact on MRSA status by independent variables that were significant ($P < 0.05$) in the univariate analyses. For age, we used the continuous variable age (in years) in our model, rather than age category. We deliberately did not include the building in our model because it covaries with room. We also did not include the number of courses of cephalexin because it covaries with the number of courses of antibiotics. Thus, the predictor variables in our multivariate analysis were age, the room in which the animal was housed, number of courses of antibiotics, the number of surgeries, SIV status, and the number of years spent in the facility.

Table 3 shows the analysis of deviance table for the multivariate analysis of the 288 animals analyzed in this cohort. Each line of the table provides a likelihood ratio test for the variable of interest, while holding the other predictors constant. Our analysis shows that the animal's room ($P < 0.0001$) was a highly significant predictor of MRSA carriage. None of the other predictors were significant.

Because the room in which the animal was housed had such a dominant effect on predicting MRSA status, and because animals in the same room often undergo the same procedures, thus increasing co-variability of room with antibiotic courses, age, time in the facility, number of surgeries, and SIV status, we performed an additional multivariate analysis that did not include room in the model. Table 4 shows the deviance table for this analysis. Using the Type II option, we found that AGE was a highly significant predictor of MRSA status ($P < 0.0001$).

Table 2. Univariate Logistic Regression Models for MRSA

Variable	Coefficient	± SE	Odds Ratio	95% CI	*Likelihood Ratio*	*P* value
AGE	0.1670	0.0397	1.18	(1.1, 1.28)	19.47	**< 0.0001**
AGE_categ	—	—	—	—	68.76	**< 0.0001**
Young/Mid	—	—	2.42	(0.89, 6.62)	3.21	0.0761
Mid/Old	—	—	7.08	(3.58, 14.02)	37.54	**< 0.0001**
Young/Old	—	—	17.16	(6.9, 42.68)	58.54	**< 0.0001**
Room						**< 0.0002**
SEX	−0.2079	0.2630	0.81	(0.48, 1.36)	0.62	0.4291
Building	—	—	—	—	30.19	**< 0.0000**
SIV	0.8990	0.2999	2.46	(1.36, 4.42)	8.77	**< 0.0001**
# surgeries	0.2100	0.0745	1.23	(1.07, 1.43)	7.95	**< 0.0001**
# courses antibiotics	0.1429	0.0443	1.15	(1.06, 1.26)	10.69	**< 0.0001**
# cephalosporin treatments	0.3186	0.0794	1.38	(1.18, 1.61)	17.53	**< 0.0001**
# baytril treatments	0.0767	0.0995	1.08	(0.88, 1.31)	0.58	0.441
# other antibiotic treatments	0.1531	0.1159	1.17	(0.93, 1.48)	1.74	0.1866
Time in facility	0.1536	0.0626	1.17	(1.03, 1.33)	6.19	**< 0.0001**

This analysis included 288 macaques. SE is the standard error, OR is the *odds ratio*, 95% CI is the 95% confidence interval.

Table 3. Multivariate Logistic Regression for MRSA

Variable	Chi Squared Likelihood Ratio	Degrees of Freedom	*P* value
Age	0.25	1	0.6402
Room	119.41	21	**< 0.0001**
# courses antibiotics	0.11	1	0.7401
# surgeries	0.22	1	0.6402
SIV	0.00	1	1
Facility Time	2.19	1	0.1386

This analysis included 288 macaques.

SIV status was also significant in predicting MRSA status ($P = 0.0111$), and the number of courses of antibiotics approached significance ($P = 0.0578$). Follow up analyses showed no significant interactions among predictors in the multivariate analyses.

Comparison of MRSA carriage and variables from 2015 and 2018 after initiating antimicrobial stewardship practices. The entire colony ($n = 285$ macaques) was surveyed again in 2018 for MRSA carriage. The MRSA carriage was 9% (26 of 285) compared with the 2015 rate of 28% (82 of 298) (Figure 2 A). Nasal culture results were compared for macaques present in the colony at both the 2015 and 2018 time points ($n = 38$) (Table 5). Results showed that 55% (21 of 38) of the animals present at both time points carried MRSA in 2015 as compared with only 18% (7 of 38) in 2018 ($P = 0.002$), with an Odds Ratio of 0.19 and relative risk of 3. Of the 21 animals that were positive in 2015, 81% (17 of 21) went from being MRSA carriers to being MRSA negative in 2018 and 19% (4 of 21) remained MRSA carriers. Furthermore, only 3 animals that were negative for MRSA in 2015 became MRSA carriers in 2018. Of these 38 macaques, those positive for MRSA in 2018 had received more courses of antimicrobials since 2015 ($n = 5.0 \pm 2.9$ SD) than had those that were MRSA negative in 2018 ($n = 1.7 \pm 3.2$ SD, $P = 0.001$) (Figure 2 B).

A similar pattern also existed for macaques that were only present in either 2015 ($n = 260$) or 2018 ($n = 247$). Among macaques that were only present in 2015, MRSA carriage was 23.8% (62 of 260), whereas among those that were only present in 2018, the MRSA carriage rate was 7.7% (19 of 247). The MRSA prevalence in these 2 groups was significantly different ($P < 0.0001$). Furthermore, animals in the 2015 survey had received more courses of antimicrobials (5.1 ± 2.7 SD) than had those in the 2018 survey (1.1 ± 1.5 SD, $P < 0.0001$), even though the 2015 animals had experienced fewer surgeries (1.9 ± 1.7 SD) than had the 2018 macaques (4.7 ± 4.7 SD) ($P < 0.0001$). The 2015 animals were also slightly but statistically younger (5.0 y ± 3.2 SD) than the 2018 animals (5.2 y ± 2.0 SD, $P < 0.0001$) (Figure 2 C).

Table 4. Multivariate Logistic Regression for MRSA without consideration of Room

Variable	Chi Squared Likelihood Ratio	Degrees of Freedom	*P* value
Age	15.58	1	**< 0.0001**
# courses antibiotics	3.60	1	0.05783
# surgeries	1.92	1	0.16638
SIV	6.45	1	**0.01109**
Facility Time	2.67	1	0.10224

Discussion

We screened 298 macaques in 2015 and determined a MRSA prevalence of 28% in a population of rhesus and pigtailed macaques housed in a research facility; after implementation of antimicrobial stewardship practices, we observed only a 9% MRSA carriage prevalence (26 of 285) in 2018. Our initial prevalence of MRSA carriage appears higher than the prevalence of MRSA in macaques reported in the limited published data available from other macaque populations (5.5.%,[51] 6.3%,[26] 17.6%,[85] and 22%,[39]), although after we implemented stewardship practices, our prevalence was much lower and more consistent with the previous reports. In another study, 39% of macaques were colonized with *S. aureus* in their nares, but MRSA was not identified.[90] The rates of carriage we observed appear higher in macaques than for the general human population (1% to 2%),[75] even for some high-risk populations such as healthcare workers (approximately 5%),[3] although other human populations may have similar rates or even higher rates (for example, in patients in intensive care, long term care, or hospitals).[64,59] Thus, by identifying the risk factors associated with MRSA carriage, we developed mitigation strategies and used antimicrobial stewardship practices[4,5] that successfully reduced MRSA in a population of macaques.

Our multivariate analysis from the initial cohort of animals identified several risk factors associated with MRSA carriage in macaques; these included the age of the animal, the room the animal was housed, and SIV/SHIV infection status. These findings partially agree with a previous study that identified veterinary and experimental interventions, as well as administration of antibiotic or corticosteroid, as risk factors of MRSA colonization of macaques; however, that study did not find age to be a risk factor.[26] Our multivariable analysis did not find that the number of antibiotic courses significantly affected MRSA prevalence. This result differs from that found in the previously mentioned study on macaques[26] and in humans, for whom the use of antibiotics is clearly a risk factor for MRSA colonization.[86] In our study, however, antibiotic use was very common in the facility in 2015. Most macaques had therefore undergone antibiotic treatment, often multiple courses; the relatively low number of animals with no antibiotic exposure perhaps reduced the overall variability of effects of antibiotic exposure across the study as a whole and thus perhaps masked significant effects of antibiotic exposure on MRSA prevalence. Nonetheless, we implemented antimicrobial stewardship practices and saw a drastic reduction in the prevalence of MRSA carriage in 2018.

One of the antibiotic treatments that most animals had received was a multimodal "clean-up" regimen of antimicrobials that included enrofloxacin and paromomycin at the time of entry into the facility, as described elsewhere.[7] This "clean-up" practice is concerning to many veterinarians in the field for multiple reasons.[8] In humans, the use of fluoroquinolones is a risk factor for MRSA colonization.[47,86,97] We would therefore expect this treatment to drive antibiotic resistance. Although our multivariate analysis did not identify the number of antimicrobial courses as a clear risk factor for MRSA carriage, most of the macaques in the 2015 study had undergone this "clean-up" procedure. Thus, most of these macaques had been exposed to fluoroquinolone, complicating our ability to fully assess fluoroquinolones or other antibiotics as drivers of resistance.

Due to our recently published concerns regarding the 'clean-up' practice,[8] we used the data from 2015 to justify and motivate antimicrobial stewardship practices similar to recently published guidelines.[4,5] This effort included working with research personnel to reduce the use of the published 'clean-up' regimen[7]

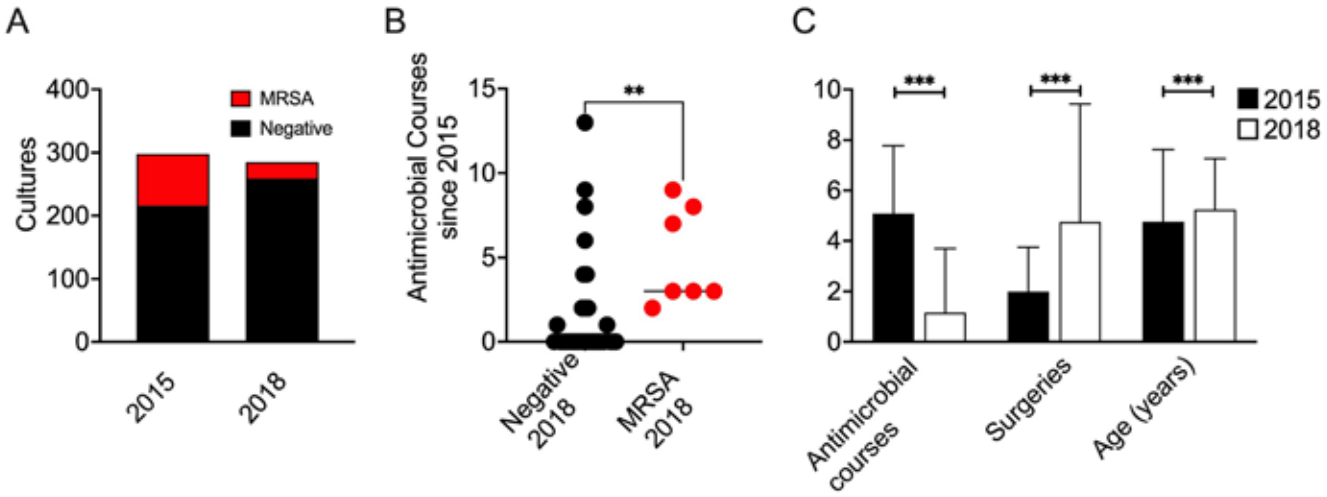

Figure 2. MRSA carriage fell between 2015 to 2018 after implementing stewardship practices. A) MRSA carriage is compared between samples collected in 2015 and 2018, MRSA carriers (red) and negative for MRSA (black). B) In macaques sampled in both 2015 and 2018, the number of courses of antibiotics since 2015 is shown for those positive for MRSA in 2018 (red) compared with those negative for MRSA (black; Mean ± 1 SD are shown, **, $P = 0.01$). C) The number of courses of antimicrobials, number of surgeries and age for animals only present in 2015 (black) or present in 2018 (white), but not both (Mean ± 1 SD are shown, ***, $P < 0.0001$).

that had been initiated in the facility years earlier, and making other changes that included reducing perioperative antibiotic use. Upon arrival to the facility, macaques that do not receive the "clean-up" regimen are almost always housed in separate rooms from those that do or have previously received it. We repeated MRSA cultures in 2018, which allowed us to compare carriage rates before and after initiation of our overall reduction in antimicrobial use across the facility. The MRSA carriage prevalence fell significantly in the population from 2015 to 2018, both in macaques that had been tested in both surveys and macaques that had been introduced into the facility since the previous survey. Thus, the earlier widespread use of antibiotics perhaps did drive antibiotic resistance, resulting in the high prevalence we saw in 2015 and the decline in 2018 after reducing antimicrobial use.

Our findings appear consistent with those reported in other species. Studies in animals and humans that implemented similar antimicrobial stewardship approaches to reduce the use of antimicrobials have shown that MRSA carriage rates may decline in response.[19,99,100] Our follow-up testing performed in 2018 showed a significant fall in MRSA carriage within the population, indicating the effectiveness of these stewardship approaches.

The strong association of MRSA nasal carriage with age in our study contrasts with some human literature that shows no association or a decrease in colonization rates with age.[16,46,64,86,93] However, some human studies do appear to show an increase in carriage with age.[80] In addition, despite some variability, both companion animals and livestock can show increased carriage rates with age, due to changes in management practices, augmented bacterial load over time, and increased human contact over time.[11,38,84,98]

We believe related risks for aged animals in our colony could involve more time for animal-to-animal spread, contact with contaminated environments, and contact with human carriers. Human data, for example, shows that environmental spread of MRSA can occur in close quarters.[34,36] This result fits with our finding that animals in certain rooms had a higher risk of MRSA carriage and with data from humans showing that exposure of humans to high-risk locations (for example hospitals, locker rooms, farms) enhances the risk of colonization with MRSA.[53,56,57] By implementing new practices to reduce antibiotic use, we could have reduced the chance of an animal developing MRSA and then exposing other macaques, thereby reducing the overall prevalence in the colony.

Humans infected with HIV are known to be at greater risk than uninfected people of being colonized with MRSA;[74] 8% to 13% of HIV-infected individuals are colonized with MRSA.[71,74] Reasons for the their increased colonization rate are likely multifactorial: increased risk of skin and soft tissue infections,[72] lifestyle risk factors,[23] prior hospitalization and antibiotic use,[74] and immunosuppression.[58] The environment of our macaques was more controlled. However, when left untreated, SIV infections in macaques result in immunosuppression and opportunistic infections.[52,82,94] However, some of the SIV-infected macaques included in our study were receiving combined antiretroviral therapy and were not necessarily immunosuppressed. However, a greater risk of MRSA carriage could occur due to SIV-related immunomodulation of its host.[18,50,91] Thus, one hypothesis that could warrant future attention is whether SIV infection alters the normal nasal mucosal immune response, the normal nasal microbiome, or both and if these factors predispose the animal to MRSA carriage.

We found that the most common genotypes of MRSA in our macaques were broadly distributed into 2 main types: sequence type 188 (ST188/*t189*) and *spa* type *t13638*. ST188 and *spa* type *t189* isolates showed significant overlap, which is consistent with prior analyses in humans[33,68] and animals.[51,73] At the time of original surveillance in 2015, ST188 was historically uncommon in humans in the Asia-Pacific area[33,55,95] but was becoming more prevalent in certain geographic areas.[96] ST188 has also

Table 5. Nasal MRSA culture results of animals present during 2015 and 2018 sample collection

	MRSA Negative: 2018	MRSA Positive: 2018	Subtotals
MRSA Negative: 2015	14	3	17
MRSA Positive: 2015	17	4	21
Subtotals	31	7	Total = 38

been identified in several different species of macaques, their environment, and in individuals working at research facilities in the USA and China.[26,51,73,85] The other common isolate that we identified was *spa* type *t13638*, which made up 59% (13 of 22) of the MRSA isolates. This isolate has also been identified in macaques in research settings in the USA[73] and China (along with *t189*).[51] This isolate appears similar to ST3268 and so far has only been reported in macaques.[26,30,51,73] These data suggest that the MRSA isolates identified in our study were probably not being introduced into the primate facilities by staff in the USA but possibly were endemic in other macaque populations. Although common human isolates (ST300) can colonize chimpanzees,[27] most of the available data from macaques suggests a different story. The finding that MRSA isolates from several species of macaques in broadly separated geographic areas were similar suggests that macaques are natural hosts to some of these isolates or that humans, or other sources, may have transmitted them to macaques many years ago, with the macaques harboring these isolates ever since. Whole genome sequencing of MRSA isolates and a more complete survey of the genotyping was beyond the scope of this work but would allow for a greater understanding of the genetic relationship of the isolates.

A limitation of our study is that we were unable to screen animal caretakers and veterinary staff for MRSA. However, if local human-macaque transmission was responsible for the carriage we have observed, we would expect that at least some of the macaques would be colonized with the more common community acquired MRSA strains (for example, ST8/USA300) circulating in the US.[22] Also, recent studies appear to suggest that humans working with macaques are colonized by similar strains to those found in the macaque populations.[30,85] Determining the sequence of carriage is difficult in such circumstances but suggests that macaques may be a potential zoonotic reservoir for certain STs of MRSA, as observed for MRSA in pigs and other livestock.[31,69,93] People working around macaques should be aware that the animals may carry zoonotic MRSA species and take appropriate precautions.

We did not attempt to eliminate carriers, despite our relatively high prevalence of MRSA carriage, as the rate of infection or complications associated with MRSA was low. Decolonization in humans is not routinely recommended due to the risk of selecting for antibiotic resistance, lack of clinical benefit, and a relatively high failure rate, especially at the population level.[37,67] Recent attempts to eliminate MRSA from colonized macaques have had some success; however, the treatment did not appear to be successful in all cases, and long-term follow-up was not described.[15,39,85] Decolonization would be a challenge in a large facility with high prevalence, as in our colony in 2015, because failure to decolonize even one animal could result in reinfection of the group and a furthering of antibiotic resistance.

Since the initial surveillance in 2015, we have implemented several practices to reduce MRSA carriage including changes in PPE practices, reducing antibiotic use, no longer using the "clean-up" protocol in most animals, and reducing the routine use of antimicrobials after surgery. This antimicrobial stewardship approach appears to have driven a reduction in MRSA carriage in our colony and should elicit caution in those who use antimicrobials at high rates in NHP populations. Recent criticism of the "clean-up" protocol has shown that this issue is not an isolated concern.[8] Future work should involve continued testing of animals to monitor for changes in prevalence or patterns, to evaluate the effectiveness of husbandry changes and antibiotic stewardship practices,[5] and to further characterize the genotypes of MRSA in this population. Screening of husbandry staff, veterinarians, and other personnel working with the primates could also help to learn more about possible zoonotic transmission.

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