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Original Research

A Simple Way to Eradicate Methicillin–Resistant *Staphylococcus aureus* in Cynomolgus Macaques (*Macaca fascicularis*)

Tae M Kim,[1,†] Hyojun Park,[2,†] Kyo W Lee,[2] Eun W Choi,[1,§] Sang H Moon,[2] Yong S Lee,[2] Kahee Cho,[3] Wan J Park,[3] Jae B Park,[2] and Sung J Kim[1,2,*]

Our investigation of indoor-housed cynomolgus macaques (*Macaca fascicularis*) by using automated identification followed by antibiotic susceptibility testing revealed 1 of 7 immunocompetent animals and 2 of 9 immunosuppressed monkeys as carriers of methicillin–resistant *Staphylococcus aureus* (MRSA). Follow-up management involving mupirocin treatment resulted in the conversion of the 3 MRSA carriers into MRSA-negative cases. Prospective assessment of newly imported monkeys involving 24-h culture of nasal swabs on chromogenic agar revealed that 22% (18 of 82 animals) were MRSA-positive. Mupirocin treatment successfully converted all of the MRSA-positive macaques into non-carriers, suggesting the feasibility of this simple, one-step screening procedure for rapidly identifying MRSA carriers in large cohorts. In addition, 8 animals that had been diagnosed MRSA-positive and subsequently treated with mupirocin demonstrated no recolonization during follow-up, even under immunosuppressive conditions. We propose rapid screening using chromogenic agar followed by mupirocin treatment as a time- and cost-effective regimen for managing MRSA in cynomolgus monkeys.

Abbreviation: MRSA, methicillin–resistant *Staphylococcus aureus*

Cynomolgus monkeys (*Macaca fascicularis*) are an exceptional animal model for various experiments in biomedical sciences and drug testing, because of their close homology with humans.[20] In general, NHP including cynomolgus macaques are not only in close contact with other animals during indoor and outdoor breeding colonies but also are frequently exposed to humans during health checks, experimental procedures, surgery, and postoperative care.[4,13] This interaction may increase the prevalence of various commensal pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA).

MRSA is a common bacteria that colonizes in nasal cavity, throat, and skin and causes several complications including systemic and wound infections in immunosuppressed hospitalized patients.[11] This pathogen was first identified among hospitalized patients in the 1960s,[3] but molecular typing has indicated that even healthy persons without healthcare-related risk factors can be carriers of MRSA, suggesting the existence of distinct community-associated strains of MRSA.[7,8] The organism is extremely difficult to manage because it frequently recolonizes after antimicrobial treatment; in addition, a new subspecies of MRSA that is resistant against vancomycin has emerged as a major concern.[11]

It is well established that MRSA colonization is recurrent in human and various animal species, including livestock.[18,25] However, little information is available regarding MRSA colonization in NHP. Notably, one study documented that 69% of chimpanzees in a captive colony were carriers, suggesting the possibility of its high prevalence in other NHP.[12] Herein, we examined the prevalence of MRSA in our colonies of cynomolgus monkeys. We also describe a MRSA screening and management procedure that can be applied to newly imported animals before handling them for experimental purposes.

Materials and Methods

Animals. Data were collected between November 2014 and March 2016. All of the cynomolgus macaques in the study originated from either Cambodia or China. All procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*[14] and the Animal Welfare Act[2] in the animal facility of the Nonhuman Organ Transplantation Research Center at Genia (Seong-nam City, Korea). Animal protocols, including the use of individual cages, were approved by the IACUC at Genia. For cross-sectional analysis, we used a total of 16 animals, comprising 7 immunocompetent and 9 immunocompromised monkeys. The subsequent prospective experimental group comprised a total of 94 newly imported macaques. Animals were individually housed indoors on a 12:12-h light:dark cycle and

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[1]Laboratory Animal Research Center, Samsung Biomedical Research Institute, [2]Department of Surgery, Division of Transplantation, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [3]Genia Inc., 143-1, Sangdaewon-Dong, Jungwon-Gu, Sungnam-Si, Gyeonggi-Do, Korea.
**Corresponding author. Email: kmhyj111@gmail.com*
[†]These authors contributed equally to this study.
[§]Current address: Department of Veterinary Clinical Pathology, College of Veterinary Medicine, Kangwon National University, Chuncheon, Korea.

were fed standard macaque biscuits (Harlan Laboratories, Seoul, Korea) and fresh fruit twice daily. Animal rooms were maintained at 23 to 25 °C and 40% to 60% relative humidity, with 15 changes of conditioned air hourly. Chlorinated, filtered fresh water was provided without restriction. All macaques were seronegative for simian retrovirus type D, SIV, simian T-lymphotropic leukemia virus, measles virus, and herpes B virus, as determined by ELISA (VRL Laboratories, Suzhou City, Jiangsu, China). *Mycobacterium tuberculosis* tests were conducted at a diagnostic laboratory (Zoologix, CA). Routine husbandry and sample collection were conducted by the same caretakers. To collect nasal swabs, macaques were held firmly while samples were collected from both nares by using Amies transport swabs (Copan Diagnostics, Murietta, CA). Skin swabs were obtained from both armpits. The swabs were then packed in an outer foil pack (VIPAK, Copan Diagnostics) and delivered to the diagnostic laboratory (SCL, Yongin-city, Gyeonggi Province, Korea) within 3 h of collection. Immunosuppressive medications used during the study period were antithymocyte globulin, cyclophosphamide, fludarabine, FK506 (tacrolimus), mycophenolate mofetil, methylprednisolone sodium succinate, and rituximab (monoclonal antibody against human CD20).

MRSA screening and decolonization management. For standard screening, swabs were streaked on blood agar plates and cultured for 24 h under aerobic conditions at 37 °C and 40% to 60% humidity, followed by gram staining, catalase testing, and coagulase testing. Gram-positive cocci that were catalase- and coagulase-positive underwent automated identification by using Vitek 2 and GP cards (bioMérieux, Durham, NC). Antimicrobial susceptibility test was conducted by using AST cards (AST-P601, bioMérieux), and MRSA status was reported as positive when samples showed resistance against cefoxitin, as indicated by the Clinical and Laboratory Standards Institute.[5] Standard screening was performed by a commercial diagnostic laboratory (SCL, Yongin-si, Gyeonggi-do, Korea).

For rapid screening, samples were obtained from both nares by using sterile dry swabs (Copan Diagnostics). The swabs were then directly streaked on CHROMagar MRSA plates (CHROMagar, Paris, France), which were cultured for 24 h under aerobic conditions at 37 °C and 40% to 60% humidity. The growth of pink to mauve colonies was interpreted as positive for MRSA. To compare the detection rate between the 2 methods, nasal samples were obtained from 17 of 82 newly imported animals by using Amies transport swabs (Copan Diagnostics). Swabs first were streaked onto CHROMagar MRSA and then were transferred into Amies transport swabs (Copan Diagnostics) and delivered to the diagnostic laboratory (SCL) within 3 h of collection.

After animals were determined to be MRSA-positive, 2% mupirocin ointment (20 mg/g, Bactroban, Hanall Biophrama, Seoul, Korea) was applied daily to the nares by using sterile dry swabs (Copan Diagnostics). Macaques were treated for 8 d in the cross-sectional analysis, whereas newly imported animals received a 14-d protocol (Figure 1). All of the animal caretakers were negative for MRSA.

Results

In our first study, we conducted a cross-sectional analysis using 16 cynomolgus macaques, 9 of which were receiving immunosuppressive drugs. Standard protocols involving biochemical and automated identification followed by antibiotic susceptibility testing

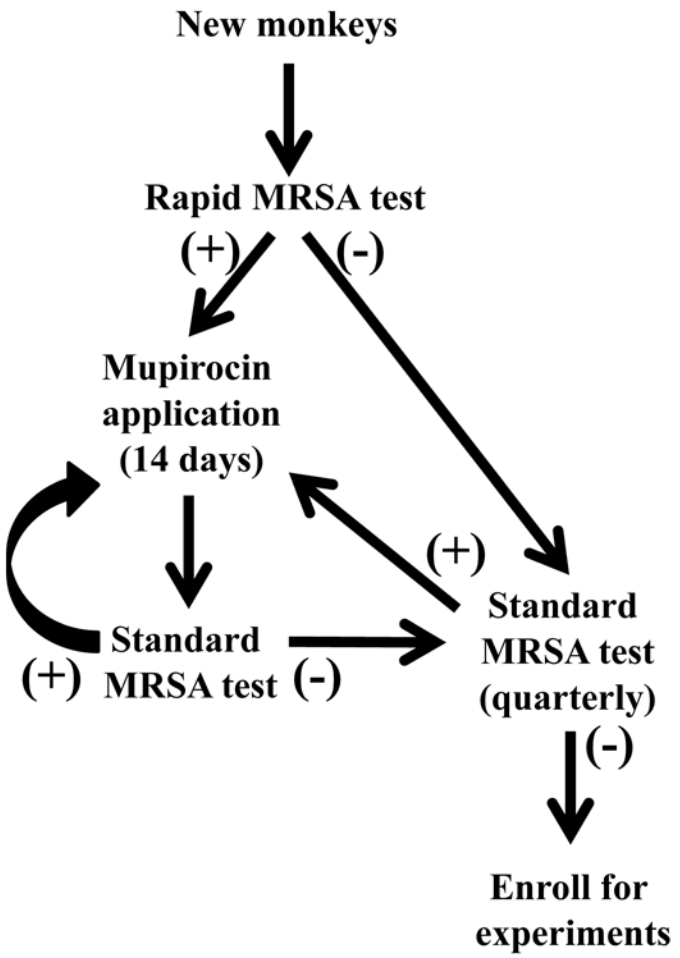

Figure 1. Our MRSA screening and treatment protocol for newly imported cynomolgus macaques. Positive animals detected by rapid culture test are treated with mupirocin daily for 14 d, and subsequent follow-up testing is performed by using the standard MRSA identification test. In cases where colonization persists, an additional round of mupirocin application is given. Negative animals undergo regular MRSA testing on a quarterly basis.

revealed that nasal swabs from 1 of the 7 immunologically normal animals yielded an MRSA-positive isolate, whereas none of the immunocompetent macaques was positive according to results from skin swabs. In addition, among the 9 immunosuppressed animals, positive isolates were identified in the nasal swabs of 2 (22.2%) and in the skin samples of 1 (11.1%). Because they yielded more robust cultures and thus were more likely to detect carriers, only nasal swabs were evaluated during subsequent testing and follow-up. We next assessed whether mupirocin ointment, which is an intranasally administrated agent for the treatment of nasal MRSA colonization in humans,[6] could be administered to these monkeys and clear them of MRSA. Mupirocin treatment led to successful eradication of MRSA in all 3 macaques (1 immunocompetent, 2 immunosuppressed). In a detailed analysis, we found that positive animals converted to negative status after the eradication processes; follow-up testing was performed 13 to 66 d after the completion of the 14-d course of mupirocin. In addition, one of the MRSA-positive immunosuppressed macaques remained positive at retesting on days 9 and 30 after mupirocin treatment;

this animal received a second course of treatment and was negative at retesting on day 66 (relative to the first course).

We then examined the prevalence of MRSA colonization in newly imported cynomolgus macaques. Although the standard screening system could have been used, we directly streaked the swab on a chromogenic agar plate, as a quick and cost-effective alternative method. This screening revealed that 22% (18 of 82) of the monkeys yielded MRSA isolates. For comparison, we used the standard protocol to retest 17 of the 82 monkeys that were tested using chromogenic agar. The 3 animals that were MRSA-positive by the standard method also tested positive by the rapid protocol (sensitivity, 100%). Among the 14 macaques that were MRSA-negative according to the standard protocol, 5 were confirmed negative by the rapid test (specificity, 36%); samples from the remaining 9 animals yielded growth.

Given the usefulness of mupirocin treatment, we then asked whether it prevents MRSA recolonization during immunosuppression with various combinations of cyclophosphamide, prednisolone, antithymocyte antibody, fludarabine, rituximab, mycophenolate mofetil, and tacrolimus. We assessed a total of 18 monkeys, 10 MRSA-negative and 8-positive according to rapid-protocol results, in this investigation. We found that mupirocin application to the 8 carriers prevented the emergence of any positive isolates according to the standard protocol; follow-up testing was performed 162 to 222 d after treatment. As expected, no MRSA-positive growth was found among the 10 animals that had been culture-negative during the preimmunosuppression period.

Discussion

The screening protocol for identifying nasal MRSA colonization that we presented in this study is a combination of 2 methods, namely standard and new, rapid protocols (Figure 1). Screening newly received NHP by using the rapid protocol facilitates the detection of carriers, thereby reducing testing burdens including turn-around time and cost.[1] Subsequent follow-up testing of our cynomolgus macaques by using the standard protocol showed that all carriers reverted to negative status after mupirocin therapy, demonstrating that this treatment regimen may prove useful for preventing MRSA recolonization even in immunosuppressed animals.

Our interest in investigating the prevalence of MRSA carriers among cynomolgus monkeys and describing an appropriate screening and eradication program is attributable primarily to the nature of our research paradigm, where transplant recipients receive various immunosuppressive regimens, thus potentially increasing the risk of recolonization and secondary infections during experimental surgeries and prolonged ICU stays.[19,21] Initial screening effort resulted in the identification of 3 positive animals among the 16 screened (18.8%), 1 from among 7 immunocompetent and 2 from among 9 immunocompromised animals. Based on these values, whether the immunosuppression protocol used in our studies is a major risk factor for MRSA colonization is unknown; rather the data clearly show the presence of MRSA in a subpopulation of the animals. Given the data obtained by using the stringent identification method that 3 among 17 (17.6%) newly imported cynomolgus macaques were MRSA-positive, we surmise that the overall prevalence of MRSA in research-naïve monkeys is approximately 20%.

We also evaluated the usefulness of our rapid MRSA identification and mupirocin treatment method for macaques receiving immunosuppressive regimes and found that 8 former carriers remained negative even after an extended period of time (9 to 173 d). However, it should be noted that only a limited number of animals was used in this study, and that data collected from serial tests under the prospective design are sparse. In addition, the effectiveness of the rapid test should be assessed carefully because its specificity is relatively low compared with other methods.[10,16] Due to these reasons, we cannot deem this method sufficient for confirmation of lack of MRSA recolonization. Furthermore, a side-by-side comparison of the 2 methods on posttreatment animals has not yet been made, and the validity of rapid culture by using CHROMagar is not fully confirmed. Nevertheless, we speculate that the rapid culture test followed by mupirocin treatment can be used as an efficient management protocol to reduce the chance of recolonization, given that positive animals were tested negative even after a substantial period. Further optimization should be made to enhance the specificity of the assay by reducing the incubation time (for example, 18 h)[10] or by conducting other supportive tests, such as prior enrichment in selective broth or coagulase test.[16] Implementing the test under other housing conditions (that is, pair housing, where recolonization is likely more frequent) is also required to make this method more practically feasible.

Currently we are unable to describe the epidemiology that caused high rates of MRSA incidence in imported monkeys; we hypothesize that either a specific strain might naturally have existed in the original colony or might have been transmitted from caregivers at the original breeding site. A recent cross-sectional analysis on captive chimpanzees found that most isolates were strain USA300, which is predominantly identified as a community-associated strain in human infections, whereas USA100 strain, the one most frequently found in human carriers, was not found in the colony.[12] Likewise, considering the nature of protective clothing that our caretakers currently use, transmission from our animal staff during quarantine of the animals is unlikely. In addition, each animal was singly housed, preventing any contact with other animals.

To date, the prevalence of MRSA carriage in cynomolgus monkeys has not been published, albeit some groups might have discussed MRSA prevalence at academic meetings. The current lack of information may be due to investigators' unwillingness to present their results because of negative perceptions regarding their facility or management program or because of the underlying difficulties in understanding the epidemiology.[9] One study reported a case of acute necrotic stomatitis in a rhesus monkey, and the authors suggested its association with MRSA and *Enterococcus faecalis* in light of culture and identification results.[15] Although the primary cause of this destructive lesion was not identified,[15] other reports highlight the possible risk of MRSA infection in animals undergoing surgery or under immunocompromising circumstances, such as social stress, chronic morbidity and immunosuppression in transplant recipients.[17,22] Other relevant work involving rhesus macaques demonstrated that 39% of animals were positive for *S. aureus* according to results of nasal swab culture, indicating that this NHP species is a natural host of *S. aureus*.[23,24] One noticeable finding from the reported study was that the strains isolated from the rhesus macaques were distinct from the ones isolated from humans in many biochemical aspects, suggesting that the colonization did not originate from humans. Together with the results from the previous chimpanzee study,[12] it seems rational to hypothesize that these NHP species might

have developed host-specific lineages distinct from those found in human carriers.

We conclude that the MRSA status of cynomolgus monkeys can easily be screened and that mupirocin application may prove useful in clearing the infection and protecting against recolonization. We believe that our current findings provide information that will be helpful for enhancing NHP management programs.

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