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VETERINARY MICROBIOLOGY - RESEARCH PAPER

Staphylococcus nasal colonization in three species of non-human primates

Juliana Georg da Silva[1] · Glenda Culuchi[1] · Cristiane Pinheiro Pestana[1] · Haroldo Cid da Silva Junior[1] · Felipe Betoni Saraiva[1] · Tatiana Kugelmeier[2] · Daniel Rouede[2] · Ana Cristina Araújo Pinto[2] · Thalita Pissinati[2] · Ana Paula D'Alincourt Assef[3] · Claudio Marcos Rocha-de-Souza[3] · Thamirys Rachel Tavares e Oliveira[3] · José Procópio M Senna[1]

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Abstract

Bacterial nasal colonization is common in many mammals and *Staphylococcus* represents the main pathogen isolated. *Staphylococcus* nasal carriage in humans constitutes a risk factor for *Staphylococcus* infections pointing out the need for animal experimentation for nasal colonization studies, especially for vaccine development. A limitation in addressing this hypothesis has been a lack of appropriate animal model. Murine models do not mimic human nasal colonization studies. Non-human primates (NHP) remain the best classical models for nasal colonization studies. In this study, we analyzed nasal colonization between two species of Old World monkeys (cynomolgus and rhesus) and a New World monkey (squirrel monkey) from breeding colony at Fiocruz (Brazil). Sixty male and female NHP with the average age of 1–21 years old, comprising twenty animals of each species, were analyzed. Nine different *Staphylococcus* species (*S. aureus*, *S. cohnii*, *S. saprophyticus*, *S. haemolyticus*, *S. xylosus*, *S. warneri*, *S. nepalensis*, *S. simiae*, and *S. kloosi*) were identified by MALDI-TOF and 16S rRNA gene sequence analyses. Antibiotic resistance was not detected among the isolated bacterial population. *S. aureus* was the main isolate (19 strains), present in all species, predominant in cynomolgus monkeys (9/20) and squirrel monkeys (7/20). *spa* typing was used to examine the clonal structure and genetic profile of *Staphylococcus aureus* isolates. Eight (8) spa types were identified among the *S. aureus* strains. A major cluster was identified, corresponding to a new *spa* type t20455, and no *spa* types found in this study were seen before in Brazil.

Keywords *Staphylococcus* spp. · Non-human primates · Nasal colonization · Animal model

Introduction

Bacterial nasal colonization is a common feature for most mammals, including rodents and non-human primates (NHP). The high prevalence of *Staphylococcus aureus* nasal carriage in humans can be considered a risk factor for *S. aureus* infections [1]. Longitudinal studies show that about 20% (range 12–30%) of individuals are persistent *S. aureus* nasal carriers, approximately 30% are intermittent carriers (range 16–70%), and about 50% (range 16–69%) are noncarriers in humans [2]. However, van Belkum et al. proposed that there are only two types of nasal carriers: persistent carriers and others [3]. Although there are studies about nasal colonization in humans [4], animal models are indispensable for the development of vaccines. Among these models, murine do not represent a suitable model for nasal colonization studies, since these animals

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✉ José Procópio M Senna
jprocopio@bio.fiocruz.br

[1] Instituto de Tecnologia em Imunobiológicos–Bio-Manguinhos/Fiocruz, Avenida Brasil 4365, Pavilhão Rocha Lima, sala 412, Rio de Janeiro, RJ CEP21040-900, Brazil

[2] Laboratório de Pesquisa em Infecção Hospitalar do Instituto Oswaldo Cruz (LAPIH/IOC/Fiocruz), Rio de Janeiro, Brazil

[3] Serviço de Criação de Primatas não Humanos do Instituto de Ciência e Tecnologia em Biomodelos (ICTB/Fiocruz), Rio de Janeiro, Brazil

are not natural carriers of *Staphylococcus* sp. In addition, the side-by-side comparison anatomical and histological differences between mice and humans are significant and display distinct morphologies attributable to the structural differences of the oral-nasal cavity: (i) the rodent innate response is remarkably different to the presence of *Staphylococcus* sp. and also resistant to nasal colonization; and (iii) longitudinal studies to establish correlations between the presence or absence of *Staphylococcus* sp. associated with immune factors would not be possible in reason of difficult area to swab; thus, many injuries can occur during the procedure leading to the sacrifice of animals [5].

Gonzalez-Zorn et al. developed a murine nasal colonization model, where after nasal inoculation of a high inoculum of methicillin-resistant *Staphylococcus aureus* (MRSA), animals eliminated the bacteria after 14 days [6]. These aspects are less than ideal for tracking *Staphylococcus* sp. nasal carriage rate and correlating individual innate responses with bacterial load over time as can be done with human studies. Non-human primates (NHP) are the closest species to humans, better mimetizing diverse responses and physiological behavior. The nasal cavity of NHP is anatomically and histologically similar to that of humans, being for this reason more indicated for studies involving bacterial nasal colonization using animals as model. Humans and monkeys have similar nasal cavity structures, such as (i) a relatively wide vestibule; (ii) the absence of a septal window, and (iii) Steno's glands; (iv) nasopharynx tonsillar tissue is also present in humans and monkeys and absent in rodents; (v) transverse lamina and (vi) olfactory recess are present in humans and monkeys and absent in rodents [7].

In addition, the investigation of nasal colonization can be used to evaluate the sanitary quality of animals and investigate the presence of pathogenic bacteria or even carriers of antimicrobial resistance profiles of animals intended for animal experimentation. Van den Berg et al. demonstrated that rhesus monkeys are natural hosts of *Staphylococcus aureus* [8], but little is known about colonization in captive breeding NHP. MRSA, a multidrug-resistant bacterium, was detected in monkeys at the Washington National Primate Research Center, in USA [9], demonstrating that NHP can be also colonized by multidrug-resistant bacteria.

In the current study, we investigate nasal colonization by *Staphylococcus* sp. in NHP from Fiocruz monkey colony. The results obtained indicate that the rhesus, cynomolgus, and squirrel monkeys could be used as animal models to study *Staphylococcus* sp. nasal colonization and vaccine development. In addition, these protocols should be applied in strategic surveillance of *Staphylococcus* sp. epizootics in non-human primates.

Material and methods

Animal study design

A total of sixty male and female non-human primates with the average age of 1–21 years old were analyzed: 20 cynomolgus monkeys (*Macaca fascicularis*), 20 rhesus monkeys (*Macaca mulatta*), and 20 squirrel monkeys (*Saimiri sciureus*). Animals were housed in groups at different enclosures with 7.35 m^2, at five per cage (squirrel monkeys and cynomolgus monkeys) and with 18 to 64 m^2, 5 to 18 animals for rhesus monkeys, receiving food twice a day and water *ad libitum*. The animals are maintained according to national [11] and international [10, 12] guidelines [, , 10–12]. The scientific farm has authorization for use and management (n° IN-052021), and the procedures are approved by the Commission of Ethics in the Use of Animals (CEUA)/Fiocruz, under license n° LW05/16. The breeding system is outdoor, in collective enclosures, where multi-male/multi-female social groups or groups of only males or females are housed. All enclosures have an external area, shelter, and anteroom. The enclosures are equipped with feeders, semi-automatic drinkers, furniture, and items for environmental enrichment. Each species is kept in different areas, forming separate colonies. The *S. aureus* colony is distributed in 6 modules with 2 rooms each (A and B), making a total of 12 rooms with 20 m^2, in a semicircle format. Each enclosure accommodates from 4 to 15 individuals. The *M. mulatta* colony is located next to *S. aureus* and has 10 modules with 2 rooms each (A and B) and 2 modules with 4 rooms (A, B, C, and D). The larger enclosures (70 m^2) house between 20 and 35 animals, while the smaller ones (35 m^2) accommodate 5 to 10 animals. The 12 modules are arranged in 4 rows of 3 modules each. The *M. fascicularis* colony has five enclosures of 40 m^2 distributed in a row, in a single construction, with 10 to 15 individuals in each enclosure. This colony is located at the back of the *M. mulatta* colony. The distance between the *S. aureus* colony and *M. mulatta* is approximately 9 m, 25 m between *M. mulatta* and *M. fascicularis*, and 63 m between *M. fascicularis* and *S. aureus*. All colonies are separated by a physical barrier (fencing). The daily handling of the animals is carried out by exclusive teams, avoiding cross flows and risks of contamination between colonies of different species. The environment is cleaned daily by collecting solid waste followed by washing with pressurized water. Twice a week, cleaning is also carried out with neutral detergent and sanitizers (e.g., 1% sodium hypochlorite solution, Virkon® S). Nutritional management is based on two food groups, industrialized feed (dry feed), which forms the basis of the diet, and natural fruit and vegetable foods and grains (wet feed). The diet

is formulated according to the nutritional needs of each species and balanced according to the physiological state of the individual or group of individuals. The dry feed is offered twice a day and the wet feed is offered at the end of the morning or the beginning of the day. The service also maintains an insectarium for the production of mealworm larvae (*Zophobas morio*) to be offered to squirrel monkeys, *in natura* or dried. Water is available *ad libitum* through semi-automatic stainless steel drinkers. All animals are monitored daily by a multidisciplinary team (veterinarians, biologists, zootechnicians, and technicians). Annual medical-zootechnical management is carried out for complete clinical and biometric evaluation and collection of biological samples for complementary laboratory tests. Animal health monitoring is also carried out, in accordance with the guidelines of the FELASA (Federation of European Laboratory Animal Science Associations). The service also has a properly equipped operating room and equipment for radiology and ultrasound.

Material collection and sample processing

The anesthetic protocol was performed by the association of ketamine hydrochloride at 10 mg/kg (Vetarnacol, König, Argentina) and midazolam at 0.1 mg/kg (Cristália Produtos Químicos e Farmacêuticos Ltda., São Paulo, Brazil) applied intramuscularly. Under mild sedation, two nasal specimens were collected with the aid of sterile cotton swabs per animal, one from each nostril.

After collection, the nasal swabs were cultured in Luria broth (LB) agar plates 3% NaCl, in order to allow the selection of Gram-positive bacteria. Plates were incubated for 18 to 24 h at 37 °C, and the resulting colonies were incubated in LB at 37 °C with agitation (120 rpm) until getting turbid. Samples were diluted 1:1 with glycerol 80% and stored at −70 °C.

Bacterial identification

Bacteria from stocks were plated in LB and incubated 37 °C for 24 h. Isolated colonies were submitted to Gram staining for light microscopy observation, to evaluate bacterial morphology. After, catalase test was performed for genus-level identification (*Staphylococcus* sp.). Fresh colonies were further used for bacterial identification MALDI-TOF-MS and 16S rRNA.

MALDI-TOF-MS analysis

All the colonies obtained from nasal swabs were submitted to MALDI-TOF-MS bacterial identification. *Staphylococcus* sp. colonies were examined in quadruplicate by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to identify the isolates to the species level and assess their genetic relatedness. The measurements were performed with MALDI Microflex LT (Bruker Daltonics, Bremen, Germany). In order to increase spectrum quality, proteins were extracted by using an extended direct transfer method that included a formic acid overlay. In detail, single colonies from freshly grown isolates were picked and directly spotted on a 96-spot steel MALDI target plate. Afterward, 1 µL of 70% formic acid (PENTA, s.r.o. Radiová, Prague, Czech Republic) was applied to each target and was left to air-dry at room temperature. One microliter of a saturated solution of α-cyano-4-hydroxycinnamic acid (HCCA) (Bruker Daltonics) was applied and allowed to co-crystallize at room temperature. Protein profiles were acquired using linear positive mode analysis with laser frequency at 20 Hz. Raw spectra were automatically acquired with the AutoXecute control software (Flex Control 3.4; Bruker Daltonics) and were recorded within the range of 2000–20,000 Da. The identification was performed using the MALDI Biotyper software (version 4.0) with default parameters, and the acquired spectra were compared with the mass-spectrum library (v6.093 MSPs). The method was externally calibrated using the Bruker Bacterial Test Standard (BTS), a manufactured extract of *Escherichia coli* DH5 alpha peptide and protein profile with two additional proteins (RNase A and myoglobin), to extend the upper boundary of the mass range covered by BTS. Results were classified using the modified score values as proposed by the manufacturer (range 2.00 to 3.00). A MRSA strain was used as positive control. Bacteria with yellow scores (1.70–1.99) and repeated results (same bacteria) were confirmed by 16S rRNA identification.

16S rRNA gene sequences analysis

For molecular characterization, DNA was extracted with the *Wizard® SV Genomic DNA Purification System (Promega Corp.)* according to the manufacturer's instructions with few modifications, to improve cell wall lysis. Briefly, isolated bacterial colonies were grown in 1 mL of LB (OD = 0.8), bacterial cultures were harvested, pellets were incubated with 50 mL of a solution containing lysozyme (20 mg/mL), and Triton 1% for 30 min at 37 °C. Identification at the species level was performed by the rrs gene sequencing, with primers for the conserved region within 16S rDNA gene. Both forward and reverse have been described previously FD1 (5′-AGAGTTTGATCYTGGYTYAG-3′) and RP2 (5′-ACGGCTACCTTGTTACGACTT-3′) [13]. The Platinum Taq DNA Polymerase High Fidelity (Thermo Fisher Scientific, Waltham, MA, USA) was used for all PCR reactions according to the manufacturer's instructions. PCR products were purified and directly sequenced in both directions by the Sanger method [14] using BigDye™ Terminator 3.1

Cycle Sequencing Kit, as described by the manufacturer (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing reactions were conducted on ABI 3500 XL Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA), and DNA sequence data were stored and analyzed with DNA Star Software Laser gene (version 5.05; DNASTAR®). A MRSA strain was used as positive control.

Antibiotic susceptibility

Staphylococcus sp. isolates were submitted to the antimicrobial susceptibility test by antibiotic disk diffusion method according to EUCAST/BrCAST, 2018 [15]. Briefly, an inoculum corresponding MacFarland 0.5 (approximately 10^5 CFU) was spread in Mueller-Hinton plates, diffusion disks containing antibiotics were placed, and plates were incubated overnight at 37 °C. Inhibition halos were measured and classed as susceptible, intermediate, or resistant.

Antimicrobial drugs (Liofilchem®) representative of the main different classes were used, including β-lactams (amoxicillin, cefoxitin, oxacillin, and penicillin), macrolides (erythromycin), lincosamides (clindamycin), chloramphenicol, quinolone (pefloxacin), tetracycline, aminoglycosides (gentamicin, kanamycin, and tobramycin), linezolid, sulfamethoxazol+trimethoprim, and glycopeptides (vancomycin). *Staphylococcus aureus* ATCC29213 (oxacillin sensitive) was used as quality control.

S. aureus molecular typing (*Spa* genotyping)

Spa genotyping PCR for amplification of the *S. aureus* protein A (*spa*) repeat region was performed according to a published protocol [16], for genetic lineage identification of the bacterial samples. The X region of the *spa* gene was amplified by PCR with primers 1095F (5′-AGACGATCC TTCGGTGAGC-3′) and 1517R (5′-GCTTTTGCAATGTCA TTTACTG-3′). DNA was obtained from isolated *S. aureus* colonies according to the extraction protocol previously described. PCR products were submitted to gel electrophoresis to evaluate purity and estimate the amplicon size. DNA sequences were obtained with an ABI 377 sequencer (Applied Biosystems, Foster City, Calif.). The forward and reverse sequence chromatograms were analyzed and compared with previous sequences from the Ridom StaphType™ (Ridom GmbH, Muenster, Germany). The identified and reliable sequences were assigned a numerical code by the specific spa type repeats found. All the FASTA sequences obtained after sequencing were aligned by MegAlign Pro with Omega Clustal (DNA Star, Inc.). The dendrogram construction and cluster analysis were obtained using neighbor joining BIONJ method. One clone of MRSA strain was included as a control.

Results

Comparison of three NHP species

The sixty healthy monkeys were randomly divided into three experimental groups, each with 20 monkeys (cynomolgus, rhesus, and squirrel monkeys). Characteristics of these animals are shown in Table 1.

Bacterial identification

After culturing each swab on a plate, the resultant colonies were observed. Colonies with different morphologies were stocked as described for further analysis. We identified 62 bacteria in the 60 animals participating in the study. In most cases, we find colonies in both nostrils. Animals AF2, AF4, AL97, and PB20 have only one nostril with colonies. Two animals (rhesus B127 and AF88) showed colonization by more than one type of colony which were further identified as *S. saprophyticus* and *S. xylosus* (AB127) and *S. nepalensis* and *S. xylosus* (AF88). These results are shown in Table S1.

All the 62 isolated bacteria were Gram-positive at microscopy observation and catalase-positive, confirming they belong to *Staphylococcus* genus.

MALDI-TOF analysis was able to identify fifty-seven (57) samples. Five (5) bacteria that do not reach the best MALDI-TOF score were confirmed by 16S rRNA sequencing. Two (2) animals presented more than one *Staphylococcus* sp., and all of them were identified. Nine (9) different *Staphylococcus* species were identified colonizing nasal cavity of the NHP in this study. (*S. aureus*, *S. cohnii*, *S. saprophyticus*, *S. haemolyticus*, *S. xylosus*, *S. warneri*, *S. nepalensis*, *S. simiae*, and *S. kloosi*). *S. aureus* was the predominant specie, being present in the three NHP lineages with 19 isolates. It was also predominant in *Macaca fascicularis* (8) and *Saimiri sciureus* (7). The second more identified specie was *S. cohnii*, with 14 isolates, being found in all the three NHP strains. Rhesus animals presented the highest variability, with eight (8) distinct species of *Staphylococcus* isolated. Six (6) distinct species were identified in squirrel monkeys and five (5) in cynomolgus macaques. These results are described in Tables 1 and 2.

Antibiotic susceptibility

Antibiotic susceptibility test was done in all the 62 *Staphylococcus* sp. samples identified. No antimicrobial resistance profile was observed in the analyzed samples. One strain

Table 1 Selected animals by species, age, sex, isolated bacteria, and antibiotic profile

Code	Cage	Age (years)	Sex	Isolated bacteria	Antibiotic profile
Cynomolgus					
AI1	1	4	M	*S. aureus*	**S**
AH19		4	M	*S. cohnii*	**S**
AJ1		2	M	*S. cohnii*	**S**
AG13		-	M	*S. haemolyticus*	**S**
O4	2	21	F	*S. aureus*	**S**
AF2		7	M	*S. warneri*	**S**
AL03		1	M	*S. aureus*	**S**
AG12		5	F	*S. saprophyticus*	**S**
Z4	3	13	F	*S. aureus*	**S**
X8		13	F	*S. cohnii*	**S**
AH6		4	F	*S. cohnii*	**S**
AD10		8	F	*S. cohnii*	**S**
X6	4	13	F	*S. cohnii*	**S**
AG2		6	F	*S. aureus*	**S**
AF4		5	F	*S. aureus*	**S**
AH8		4	F	*S. aureus*	**S**
AI2	5	3	F	*S. aureus*	**S**
AG4		6	F	*S. aureus*	**S**
AB2		11	F	*S. cohnii*	**S**
AG10		8	F	*S. saprophyticus*	**S**
Rhesus					
AL23	1A	6	M	*S. xylosus*	**S**
AL97		5	M	*S. cohnii*	**S**
Q63	2A	23	M	*S. aureus-*	**S**
AE118		11	F	*S. cohnii*	**S**
AL90		5	F	*S. xylosus*	**S**
AD52		12	F	*S. cohnii*	**S**
Z108		16	F	*S. haemolyticus*	**S**
AB29	1B	14	M	*S. haemolyticus*	**S**
AC85		13	M	*S. kloosi*	**S**
AG75		10	M	*S. warneri*	**S**
AA122	5B	15	F	*S. haemolyticus*	**S**
AB127		14	M	*S. saprophyticus/S. xylosus*	**S**
AD47		12	M	*S. aureus*	**S**
AF88		10	F	*S. xylosus/S. nepalensis*	**S**
T24		21	F	*S. saprophyticus*	**S**
V52	8A	18	F	*S. haemolyticus*	**S**
AC19		14	M	*S. haemolyticus*	**S**
AN4		3	F	*S. warneri*	**S**
AP19		2	M	*S. aureus*	**S**
AL44		5	F	*S. warneri*	**S/I***
Saimiri					
PB4	1A	10	F	*S. saprophyticus*	**S**
PF2		5	F	*S. cohnii*	**S**
PB11	2A	10	M	*S. aureus*	**S**
PC7		8	M	*S. aureus*	**S**
V13	4A	11	M	*S. aureus*	**S**
PE32	5A	6	F	*S. cohnii*	**S**
PF14		5	F	*S. simiae*	**S**
PA46	1B	11	F	*S. saprophyticus*	**S**
PA8		11	F	*S. saprophyticus*	**S**
AC61	3B	6	M	*S. cohnii*	**S**
AA21		8	M	*S. saprophyticus*	**S**
5501		19	M	*S. aureus*	**S**
56	4B	16	F	*S. aureus*	**S**
5548		16	F	*S. kloosi*	**S**
PD4	5B	7	F	*S. saprophyticus*	**S**
PB20		10	F	*S. aureus*	**S**
PE19	6B	6	M	*S. cohnii*	**S**
PB63		9	M	*S. aureus*	**S**
PD19		7	M	*S. nepalensis*	**S**
PF27		5	M	*S. simiae*	**S**

M, male; *F*, female; *S*, sensitive; *I*, intermediate

*Strain presenting intermediate resistance to SXT (sulfametoxazol-trimetoprim) and sensitive to all other antibiotics

(*Staphylococcus warneri*, strain AL44, isolated from the animal rhesus AL44) presented intermediate resistance to SMT-TMP. These results are represented in Table 1.

Spa genotyping

Spa type was used to evaluate the presence or not of clonal *S. aureus* strains among the 19 isolates from the NHP in this study. All sequence repeats were identified in the Ridom

Table 2 Bacteria isolated from non-human primates*

Bacteria (total)	Cynomolgus	Rhesus	Squirrel monkeys
S. aureus (19/30.7%)	9	3	7
S. cohnii (14/23.3%)	7	3	4
S. saprophyticus (9/15%)	2	2	5
S. haemolyticus (6/10%)	1	5	-
S. xylosus (4/6.4%)	-	4	-
S. warneri (4/6.4%)	1	3	-
S. nepalensis (2/3.3%)	-	1	1
S. simiae (2/3.3%)	-	-	2
S. kloosi (2/3.3%)	-	1	1

*The total number of isolates was higher than the animal number because more than one bacteria was isolated from two animals (n = 62)

database. Nine (9) *spa* types were found among the isolated *S. aureus*. One cluster (*spa* type t20455), comprising nine (9) samples, was identified. This cluster is composed of bacteria isolated from seven (7) cynomolgus (AG4, O4, AG2, AI1, AI2, AL2, and AH8), one (1) rhesus (AP19), and one squirrel monkey (PB20) species, respectively. This cluster was classed as a new *spa* type and included in the Ridom database. Any of the other *spa* types identified here were previously found in Brazil. Spa type distribution is represented in the Fig. 1. Table 3 shows the spa type repeats, worldwide distribution, and frequency of each spa type identified in this study.

Discussion

Staphylococcus sp. nasal carriage considered a potential source of infections [17] in humans and susceptible animals are particularly important for studies involving new strategies for nasal decolonization for diminishing staphylococcal infections. Rodents has strong limitations concerning staphylococcal nasal colonization studies. Using non-human primates, our closest biological relatives, is urgently needed for development of models that can mimics characteristics observed in human staphylococcal infections. The nasal passages of non-human primates are anatomically and histologically similar, and reproductive mucosal studies reveal similarities in the immune responses to pathogens and human-relevant microbial profiles [5]. Originating from a common phylogenetic ancestor with humans, NHPs have served as an attractive animal to model human bacterial infections due to their nearly identical anatomy and physiology, as well as host response to infection [18].

In this study, we analyzed *Staphylococcus* sp. nasal carriage between two species of Old World monkeys (*M. fascicularis* and *M. mulatta*) and a New World monkey (*S. sciureus*) from breeding colony at Fiocruz (Brazil). Our data shows a surprising high bacterial diversity; nine different *Staphylococcus* species were identified. *S. aureus* was the predominant species identified in this study, predominant isolated in cynomolgus and squirrel monkeys and followed in rhesus macaque. Van den Berg et al. showed 39% of nasal

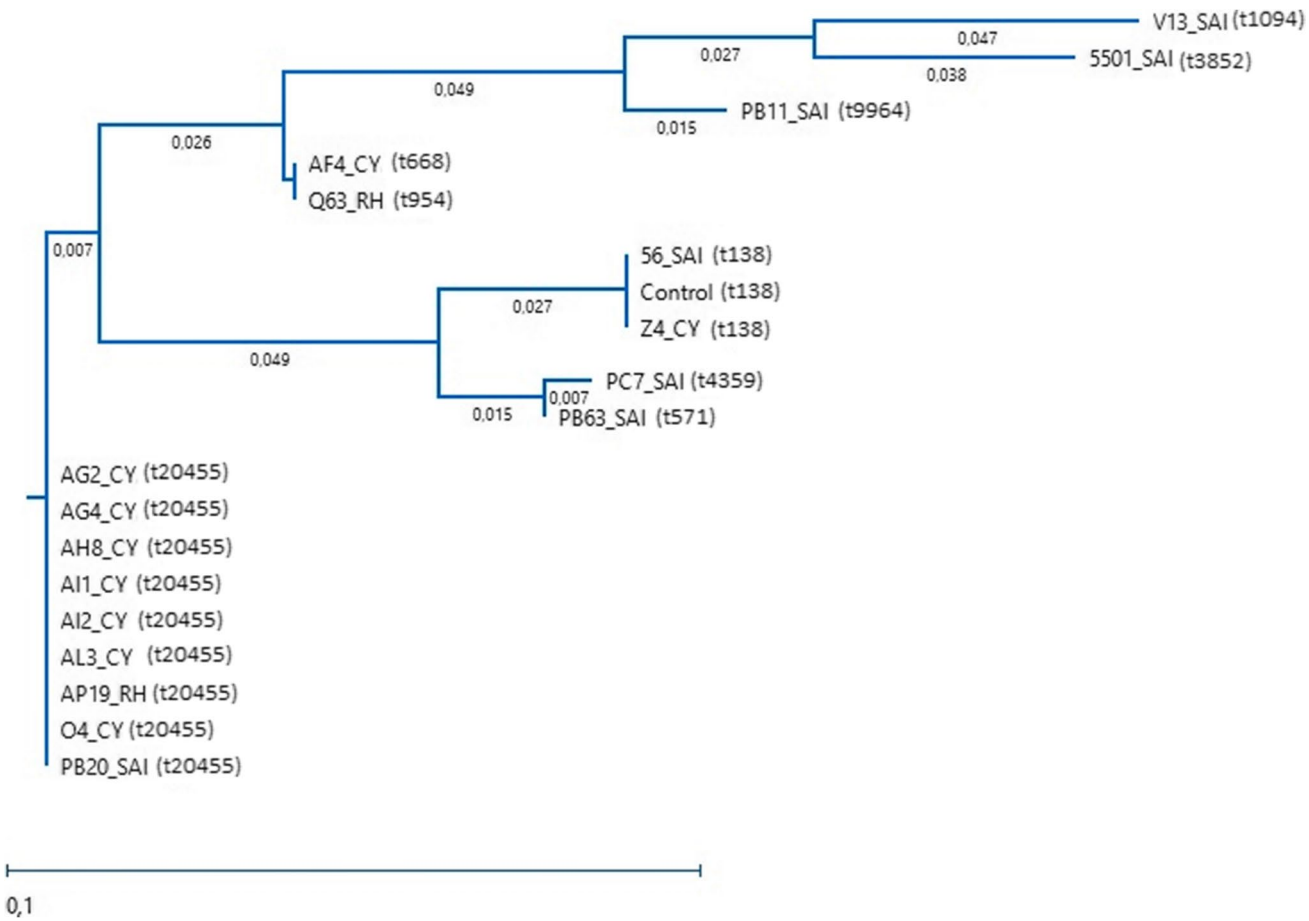

Fig. 1 Dendrogram comprising *Spa* types from all *Staphylococcus aureus* strains isolated from NHP in this study. Code number of animals and species are indicated. CY, cynomolgus; SAI, *Saimiri*; and RH, rhesus

Table 3 Representation of spa type repeats and distribution

ID	Repeats	SPA type	Distribution	%
Control (MRSA)	08-16-02-25-17-24	t138	Austria, Belgium, China, Denmark, Germany, Iceland, Ireland, Italy, Netherlands, New Zealand, Norway, Taiwan	0.03
56_SAI				
Z4_CY				
PB63_SAI	08-02–16-25-02-25-34-25	t571	Austria, Germany, Israel	0.01
AF4_CY	26-23-17-34-17-20-17-16	t668	Argentina, Germany, Iceland, Ireland, Netherlands, Norway, Spain, Sweden, United States	0.02
Q63_RH	26-23-17-34-17-17-16	t954	Germany, Sweden, Spain, Netherlands, United Kingdom, Norway, United States, France	0.03
V13_RH	23-17-34-17-20-17-12-17-16	t1094	Germany, Israel, Netherlands, United States	00.01
AP19_RH	35-17-34-17-20-17-16-16	t20455	Brazil	0.00
PB20_SAI				
AG4_CY				
O4_CY				
AG2_CY				
AI (1)_CY				
AI (2)_CY				
AL3_CY				
AH8_CY				
5501_SAI	07-23-21-17-34-13-34-33-34	t3852	Brazil	0.00
PC7_SAI	08-02-25-02-25-34-25	t4389	Germany, Poland, UK	0.01
PB11_SAI	07-23-12-21-17-12-12	t9964	not described	0.00

carriage were *S. aureus* among 731 rhesus macaques [8]. In this work, the rate of *S. aureus* isolation remained in the order 25%. Other species identified included *S. saprophyticus* as the predominant often related to infections in humans, as a common cause of uncomplicated urinary tract infections (UTIs), particularly in young sexually active females [19]. *S. cohnii*, present in 23.3% of the bacterial isolates, is considered a human skin commensal, with few reported cases of infections in humans [20, 21]. *S. haemolyticus* produce biofilms could be associated with colonization and disease among preterm neonates [22]. The other species identified (*S. xylosus*, *S. warneri*, *S. nepalensis, S. simiae*, and *S. kloosi*) are found in animal sources and rarely isolated from humans [23].

Antimicrobial resistance in animals is a major concern that have been well investigated in veterinary medicine lately [24]. In the present work, antimicrobial resistance was not seen among the selected samples, which can be considered a good indicator of the sanity and correct handling of animals. This is a critical point, since these animals will be used in trials and the spread of antimicrobial-resistant bacteria could spread to operators and have an impact on the assays involving these animals.

As *S. aureus* is the most prevalent identified strain and the most intensively studied human pathogen and commensal, we decided to investigate the clonality of the isolated samples in the three NHP species studied. Protein A, which is present exclusively in *S. aureus*, allows the use of this method for molecular typing. Previous studies showed that this method has a good correlation between clonal groupings determined by another typing method, MLST (multi-locus sequencing typing) [25], used in staphylococcal epidemiological studies. *Spa* type showed to be easy to perform, unexpensive, and rapid. This method allowed us to identify a main clone in nine (9) samples, predominant in cynomolgus and present in squirrel monkeys and rhesus species. This clonal type was classed as a new *spa* type, which was assigned for the submitted sequence: t20455 (repeats: 35-17-34-17-20-17-16-16) by the Ridom server. According to the server's data, all the *spa* types found in this study have not been registered in Brazil, so far.

In conclusion, our results indicate that all the monkeys were colonized by different *Staphylococcus* species. Since cynomolgus macaque was the most naturally colonized by *S. aureus*, we have successfully established this model as the most suitable for nasal colonization studies by these bacteria.

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Declarations

Competing interests The authors declare no competing interests.

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