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## Original article

# Methicillin-resistant *Staphylococcus aureus* nasal carriage in neonates and children attending a pediatric outpatient clinics in Brazil

Maria Aparecida Vieira<sup>a</sup>, Ruth Minamisava<sup>b,\*</sup>, Vicente Pessoa-Júnior<sup>c</sup>,  
Juliana Lamaro-Cardoso<sup>d</sup>, Yves Mauro Ternes<sup>c</sup>, Maria Cláudia Porfirio Andre<sup>d</sup>,  
Sabrina Sgambatti<sup>e</sup>, André Kipnis<sup>d</sup>, Ana Lúcia Andrade<sup>c</sup>

<sup>a</sup> Department of Nursing, Pontifícia Universidade Católica de Goiás, Goiânia, GO, Brazil

<sup>b</sup> Faculty of Nursing, Universidade Federal de Goiás, Goiânia, GO, Brazil

<sup>c</sup> Department of Community Health, Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, GO, Brazil

<sup>d</sup> Department of Microbiology, Institute of Tropical Pathology and Public Health, Universidade Federal de Goiás, Goiânia, GO, Brazil

<sup>e</sup> Department of Medicine, Pontifícia Universidade Católica de Goiás, Goiânia, GO, Brazil

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## ABSTRACT

**Background:** In Latin America, few studies have been carried out on methicillin-resistant *Staphylococcus aureus* carriage in the pediatric population. We conducted a survey of nasal *S. aureus* carriage in neonates and in children attending the pediatric outpatient clinics in a large Brazilian city with high antimicrobial consumption.

**Methods:** Pernal swabs of neonates were collected upon admission and at discharge in four neonatal intensive care units and of children less than five years of age during outpatient visits. Methicillin-resistant *S. aureus* isolates were characterized for antibiotic susceptibility, *mec* gene presence, pulsed-field gel electrophoresis, *spa* type, *SCCmec*-type, multilocus sequence type, and presence of Pantone-Valentine leukocidin genes.

**Results:** *S. aureus* was carried by 9.1% and 20.1% of the 701 neonates and of 2034 children attending the outpatient clinics, respectively; methicillin-resistant *S. aureus* carriage was detected in 0.6% and 0.2%, of the these populations, respectively. Healthcare-associated methicillin-resistant *S. aureus* strains found in neonates from neonatal intensive care units and outpatients were genetically related to the Brazilian (*SCCmec*-III, ST239) and to the Pediatric (*SCCmec*-IV, ST5) clones. Community-associated methicillin-resistant *S. aureus* was only detected in outpatients. None of the methicillin-resistant *S. aureus* strains contained the Pantone-Valentine leukocidin gene. Methicillin-resistant *S. aureus* strains related to the Brazilian clone showed multidrug resistance pattern.

**Conclusions:** Despite the high antibiotic pressure in our area, and the cross transmission of the healthcare-associated methicillin-resistant *S. aureus* clones between neonatal intensive care units and outpatients, the prevalence of methicillin-resistant *S. aureus* carriage is still low in our setting.

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\* Corresponding author at: Rua 227, Qd. 68, S/N, Setor Leste Universitário, Goiânia, Goiás 74605-080, Brazil.

E-mail address: [minamisava@gmail.com](mailto:minamisava@gmail.com) (R. Minamisava).

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## Introduction

*Staphylococcus aureus* is a common cause of community and healthcare-associated illness. Carriage of *S. aureus* is frequent in children and the anterior nares are the most consistent site for *S. aureus* colonization.<sup>1</sup> Children colonized with methicillin-resistant *S. aureus* (MRSA) are potential reservoirs for the spread of MRSA in the community.<sup>2,3</sup> The relationship between MRSA nasal carriage and invasive staphylococcal infection has been well documented.<sup>4</sup> A limited number of MRSA clonal lineages has been responsible for the majority of MRSA infections in several regions.

Since the emergence and rapid spread of community-associated MRSA (CA-MRSA) in previously healthy hosts,<sup>5</sup> attention has been given to studies reporting changes in the epidemiology of MRSA colonization especially CA-MRSA in hospitalized patients<sup>6</sup> and healthcare-associated MRSA (HA-MRSA) in healthy subjects.<sup>7,8</sup>

In neonatal intensive care units (NICUs), neonates are at high risk for MRSA infections. Patients colonized by MRSA in NICUs present significant rates of bloodstream infection<sup>9</sup> and are often transferred to other hospitals, contributing to the spread of MRSA in health facilities. Studies on MRSA carriage in neonates, however, are usually conducted under outbreak situations in NICUs.<sup>10,11</sup>

Despite the severity and rapid global expansion of MRSA strains, the epidemiology of MRSA nasal carriage remains unclear. In Latin America, few molecular studies are available on MRSA carriage in the pediatric population.<sup>12,13</sup> To determine the extent the nasal carriage of MRSA is widespread in the pediatric population, we carried out a survey in neonates and in outpatient children in a large Brazilian city. We investigated molecular characteristics of MRSA isolates.

## Materials and methods

### Study area

The study was conducted in the municipality of Goiânia, capital of Goiás state, located in Central Brazil, a region with high rates of antibiotics consumption (Andrade 2012). In the 2010 census, the population of Goiânia was estimated at 1,302,001 inhabitants, with 20,014 live births, infant mortality at 14.6 deaths per 1000 live births, and 84,465 children under five years.<sup>14</sup> Public health care is provided free of charge by the Brazilian Unified Health System, and an estimated 75% of the population use the public health system.<sup>15</sup> In Goiânia, children are first seen at the public healthcare centers (pediatric outpatient clinics) and referred to hospitalization whenever necessary. For this study, two pediatric populations were screened for MRSA, as described below.

### Neonates admitted to NICUs

From June 2007 through November 2008 were screened for MRSA carriage all neonates admitted to four NICUs, which attend children from public and private health insurance. Taking together these NICUs comprise the majority of beds ( $n = 69$ )

for neonates in the city. Data were collected on demographics (gender, date of birth, and age at NICU admission), each neonate's clinical history (length of hospital stay, congenital malformation, birth weight, preterm, timing of swab collection, nasogastric intubation, and diagnoses upon admission and at discharge), and variables related to their mothers (type of delivery, schooling, number of prenatal visits, and placental abruption).

### Outpatient children

The carriage survey was conducted from July 2007 through July 2008 in children less than five years of age attending the major outpatient clinic of the city, served by the Brazilian public health insurance (Brazilian Unified Health System). Data were collected on child (gender, age, congenital malformation, skin and soft-tissue infections, hospitalization in the last six months, antibiotic use in the last three months, day care attendance in the last six months, acute otitis media in the last 12 months, pneumonia in the last 12 months, and tonsillitis in the last 12 months) and the family (mother's schooling, smoking at home, number of family members, and health worker in the family). We calculated that a sample size of 2000 children would be necessary to ascertain MRSA prevalence assuming a 1.2% prevalence of MRSA nasal carriers<sup>16</sup> with a 95% confidence interval (95% CI), a precision of 0.7%, design effect of 1.8, and a refusal rate of 10%. The number of children who were recruited at the outpatient clinics per day took into account the laboratory capability for processing nasal specimens (approximately 10 swabs/day).

### Collection of nasal specimens

Pernasal flocked swabs were collected by rotating the swab twice in the vestibule of the anterior nostrils of each child. In the outpatients, a single sample was collected from each child. At the NICUs, two samples were collected per child; the first collected upon admission and the second at discharge or death. After collection, the swabs were placed in a liquid medium (ESwab, Copan Diagnostics, Inc., Murrieta, CA, USA) and sent to the Microbiology Laboratory of the Federal University of Goiás for immediate processing.

### Study ethics

The Institutional Review Board approval was given by the Ethics Committee of the Federal University of Goiás (# 008/2007). All parents or legal guardians signed copies of the informed consent form before recruitment.

### Microbiological techniques

The specimens were plated onto mannitol salt agar (Difco<sup>®</sup> Laboratories Inc., Detroit, MI, USA) for *S. aureus* isolation. Trypticase soy agar plates, which were supplemented with 4% NaCl and oxacillin at 6 µg/mL, were used to screen MRSA isolates. After 24–48 h of incubation at 37 °C, the colonies that were suspected of being *S. aureus* were confirmed by Gram staining,

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