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Major article

Molecular characteristics of *Staphylococcus aureus* isolated from employees, children, and environmental surfaces in Iowa child daycare facilities

Erin D. Moritz PhD *, Blake M. Hanson PhD, Ashley E. Kates MS, Tara C. Smith PhD

Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, IA

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Background: Infectious agents have the potential to thrive in child daycare facilities. Asymptomatic *Staphylococcus aureus* carriage is a risk factor for developing infection and contributes to transmission.
Methods: We collected swabs from 110 employees, 111 unexposed adults, 81 children, and 214 environmental surfaces at 11 Iowa daycare facilities. *S aureus* isolates were characterized using antibiotic resistance profiles and *Staphylococcal* protein A typing. *Staphylococcal* protein A types were grouped into cluster complexes using the Based Upon Repeat Pattern algorithm.
Results: All isolates (from 38 employees, 37 unexposed adults, 16 children, and 19 surfaces) were characterized. Daycare employees were more likely to carry erythromycin-resistant *S aureus* than unexposed adults (odds ratio, 3.7; 95% confidence interval, 1.1-12.7; $P = .033$). Isolates were genetically heterogeneous, although isolates from employees appeared more clonal than those from unexposed adults. Strains associated with ST8 were identified in 5 daycare facilities and 3 unexposed adults.
Conclusions: *S aureus* isolates collected from employees, children, and surfaces of daycare facilities are genetically heterogeneous, but contain strains associated with community-associated methicillin-resistant *S aureus*. This suggests that daycare facilities can serve as reservoirs for community-associated methicillin-resistant *S aureus* and facilitate genetic exchange. Employees may be at increased risk of carrying antibiotic-resistant strains, indicating more research is necessary into this occupational group.

Asymptomatic carriage of *Staphylococcus aureus* is an important risk factor for developing infection, as well as a key contributor to transmission.[1] A worrisome trend in *S aureus* epidemiology is the rise in frequency and severity of strains that are resistant to antimicrobial agents, particularly methicillin-resistant *S aureus* (MRSA). Although MRSA has been recognized in the health care environment since 1968, Community-associated MRSA (CA-MRSA) occurs in individuals without established risk factors for hospital-associated MRSA (HA-MRSA) (ie, little or no exposure to a health care setting).[2] The recent rise of CA-MRSA in children has raised questions specifically about *S aureus* in child daycare settings and the role of daycare facilities in community pathogen transmission as a whole.[3-5]

Infectious agents have the potential to thrive in daycare settings, where large numbers of children and employees are in close contact with one another for long periods of time. Children's behavior (eg, the tendency of children to put hands and objects in their mouths) and adults' behavior (eg, hygiene practices and close physical contact with children), as well as physiologic factors in children (eg, immature immune systems and incontinence) contribute to the success of pathogen transmission in daycare facilities.[6] Daycare facilities could serve as important community reservoirs of *S aureus*. Hewlett et al[7] reported that 90% of MRSA infections isolated from a hospital-based daycare center (18 out of 21) were classified as CA-MRSA. Moreover, several studies have identified HA-MRSA strains at daycare centers.[7-10]

* Address correspondence to Erin D. Moritz, PhD, 9315 Gaither Rd, Gaithersburg, MD 20877.
E-mail address: moritz.erin@gmail.com (E.D. Moritz).
At the time the study was conducted, EM was with Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, IA. She is currently employed in Gaithersburg, MD.
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We sought to characterize molecular properties of *S aureus* isolated from daycare employees, children, and environmental surfaces from 11 Iowa daycare facilities, with the goal of revealing potential routes of transmission.

MATERIALS AND METHODS

Sample collection and isolation

We conducted a cross-sectional study of *S aureus* in daycare facilities located in Johnson County, Iowa, between February 2009 and February 2010. Comprehensive recruitment methods are published elsewhere,[11] but involved identifying child-care centers from the Johnson County list of registered and licensed facilities, as well as through personal contacts. Letters were sent to all facilities and follow-up telephone calls were conducted, with priority given to larger licensed facilities. Age and gender frequency-matched unexposed adults were recruited at the University of Iowa College of Public Health student commons (location 1), a University of Iowa nonlaboratory/administrative building (location 2), and the University of Iowa student union on the main campus (location 3). Nasal and throat samples were collected from adults, whereas only nasal swabs were collected from children to minimize discomfort.[11] Environmental samples were collected throughout each facility, concentrating on areas that are frequently touched (eg, doorknobs and popular toys). After immersing the swab in sterile phosphate-buffered saline, samples were obtained by rotating the swab in 3 directions across as much of the surface as possible.

Samples were collected using cotton-tipped transport swabs (BD BBL Culture Swabs with Liquid Stuart media; Becton, Dickinson, and Company, Sparks, Md, and Remel, Lenexa, Kan), kept at 4°C during transport, and processed within 24 hours of collection. *S aureus* and MRSA were isolated and confirmed as previously described.[12] Swabs were inoculated into *S aureus* enrichment broth and subsequently plated onto Columbia colistin-nalidixic agar with 5% sheep blood (Columbia CNA; Remel) and selective MRSA agar plates (BBL CHROMagar MRSA, Becton, Dickinson, and Company) for identification. *S aureus* isolates were confirmed using Gram's stain, the catalase test, the slide coagulase test, and an *S aureus* latex agglutination assay (Pastorex Staph-plus; Bio-Rad, Marnes-la-Coquette, France). Methicillin resistance was identified with a MRSA latex agglutination test (Oxoid Ltd, Hants, UK) and confirmed using *mec*A polymerase chain reaction (PCR).[12,13] All samples, regardless of the source, were processed identically. A participant was considered colonized if a nose swab, a throat swab, or both yielded *S aureus*.

Adults and parents of participating children filled out a demographic/risk factor questionnaire. The questionnaire contained 47 questions addressing basic demographics, medical history, and exposures and lifestyle factors that may be associated with *S aureus* carriage. The questionnaire also inquired about exposures in immediate family members and anyone who lived in the participant's household. Daycare employees answered 6 additional questions about their employment status, the types of work routinely performed, and the numbers and ages of children with whom they have routine contact. Any employee of a facility was invited to participate; only 1 employee reported not having routine contact with children. This study was approved by the University of Iowa Institutional Review Board.

Antibiotic resistance profiles

All *S aureus* isolates were tested at the University of Iowa Hospitals and Clinics for antimicrobial susceptibility by the broth dilution method described by the Clinical and Laboratory Standards Institute.[14] Isolates were tested for susceptibility to oxacillin, tetracycline, erythromycin, clindamycin, linezolid, levofloxacin, vancomycin, trimethoprim/sulfamethoxazole, daptomycin, and quinupristin/dalfopristin.

Statistical analysis

Sample size was calculated based on the primary goal of the study, which was to detect if child-care employees are at increased risk of carrying *S aureus*, assuming 30% of unexposed control participants and 50% of child-care employees carried *S aureus*, respectively ($\alpha = .05$; $\beta = .80$). Data analysis was conducted using SAS 9.2 (SAS Institute Inc, Cary, NC). Pearson χ^2 or Fisher exact test was used to evaluate predictors of antibiotic resistance in adult participants. Multivariate modeling was performed using manual forward selection.

Molecular characterization

Sequencing and assignation of *Staphylococcal* protein A (*spa*) type was performed as described elsewhere.[15] Briefly, PCR was performed to amplify the polymorphic X region of the *S aureus spa* gene. PCR products were sequenced at the University of Iowa DNA facility using an Applied Biosystems Model 3730 DNA sequencer. The *spa* types were assigned and compared using RidomStaphType software version 2.1.1 (Ridom, Wurzburg, Germany). The Based Upon Repeat Pattern (BURP) algorithm was used to group *spa* types into cluster complexes (*spa*-CCs).[16,17] Pairwise cost distances ≤ 6 were used to define *spa*-CCs. *spa* types containing 5 or fewer repeat regions were excluded. Panton-Valentine leukocidin (PVL) gene detection was conducted using published methods.[18]

RESULTS

Prevalence of S aureus

This report contains data from 11 facilities visited between February 2009 and February 2010. Ten facilities were located within the Iowa City metropolitan area; 10 were licensed facilities (Table 1). Facility capacities ranged from 3-60 employees (mean, 21; median, 18) and from 16-168 children (mean, 53; median, 33).

One hundred ten employees participated in this study; the mean age was 29.7 years (median, 24 years; range, 16-64 years) and 92.6% (n = 102) were female. The prevalence of any *S aureus* carriage in employees was 34.5% (n = 38), whereas the prevalence of MRSA was 3.7% (n = 4). Eighty-one children participated in this study, with a mean age of 2.9 years (median, 3 years; range, 6 months-7 years). Females accounted for 57.9% (n = 47) of child participants. The prevalence of any *S aureus* in children was 19.8% (n = 16), whereas 1.2% (n = 1) carried MRSA. One hundred eleven adults not employed at a daycare facility participated; the mean age was 31.8 years (median, 27 years; range, 18-78 years) and 88.3% (n = 98) were female. The prevalence of any *S aureus* in unexposed adults was 33.3% (n = 37), whereas MRSA was carried by 0.9% (n = 1) of unexposed adults.

A total of 214 surface samples were obtained, with a mean of 20 surfaces sampled per facility, depending on center size. Overall, 8.4% (n = 18; range, 3.4%-30%) of the surface swabs were positive for any *S aureus*, and 1.4% (n = 3; range, 0%-3.7%) were positive for MRSA (Table 2). The MRSA isolates were identified in different centers: from an infant high chair, a plastic "learning walker" in an infant room, and large tool bench play set.