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Major Article

Nasal colonization of *Staphylococcus aureus* colonal complex 5: Prevalence, influencing factors, and phenotypic and molecular characteristics in pregnant Chinese women

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Staphylococcus aureus
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Background: Colonal complex 5 (CC5) has been referred to as the most pandemic community-associated *Staphylococcus aureus* in most Asian countries. However, few studies have focused on CC5 isolates in pregnant women. The aim of this study was to determine the prevalence and phenotypic and molecular characteristics of *S aureus* and methicillin-resistant *S aureus* (MRSA) CC5 nasal colonization in pregnant Chinese women.
Methods: We performed a cross-sectional study between August and November 2015 in 2 hospitals in Shenzhen, China. Pregnant women were asked to complete questionnaires, and nasal swabs were collected. Log-binomial regression models were used to explore factors influencing *S aureus* and MRSA nasal colonization between the CC5 and non-CC5 or non–*S aureus* groups. Polymerase chain reaction assays were used to detect the molecular characteristics of isolates.
Results: Overall, 2,172 pregnant women were included in this study. The prevalence of *S aureus* and MRSA was 25.60% (n = 556) and 5.62% (n = 122), respectively. The multilocus sequence typing of *S aureus* isolates was diversified. A lower frequency of daily handwashing (<7) and weekly bathing (<7) were risk factors for the prevalence of *S aureus* (adjusted prevalence ratio [aPR], 1.13; 95% confidence interval [CI], 1.03-1.41 and aPR, 1.22; 95% CI, 1.03-1.45) and MRSA (aPR, 1.96; 95% CI, 1.23-3.14 and aPR, 1.47; 95% CI, 1.21-2.44) nasal colonization in the CC5 groups of pregnant women.
Conclusions: The prevalence of *S aureus* and MRSA nasal colonization was moderate. The molecular characteristics of *S aureus* and MRSA isolates indicated possible cross-transmission among multiple resources. A higher frequency of daily handwashing and weekly bathing significantly decreased the prevalence of *S aureus* and MRSA CC5 nasal colonization in the pregnant women.

Staphylococcus aureus is a common clinical pathogen,[1] and the use of antibiotics can promote the emergence of methicillin-resistant *S aureus* (MRSA).[2] Antibiotic-resistant *S aureus*, one of the leading causes of antibiotic-resistant nosocomial infections, causes diseases that range from minor skin infections[3] to severe pneumonia[4] and endocarditis,[5] and it is of particular concern because few antibiotics are effective at treating infections caused by the pathogen. Moreover, it poses a great threat to public health globally because it can require complicated therapeutic regimens and may have poor clinical outcomes.[6]

The epidemiology of antibiotic-resistant *S aureus* infections has changed in the last decade because of the emergence of community-associated *S aureus* in patients without any risk factors or previous health care contact.[7,8] Community-associated *S aureus* isolates have unique molecular characteristics that distinguish them from health care–associated and livestock-associated *S aureus*. Colonal complex 5 (CC5) has been referred to as the most pandemic community-associated *S aureus* in most Asian countries.[9,10]

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Few reports have described the epidemiology and phenotypic and molecular characteristics of community-associated *S aureus* among pregnant women in developing countries in Asia. However, several studies have reported that MRSA is the main cause of neonatal infections[11,12] and have revealed that MRSA colonization of pregnant women may be a potential risk factor for neonatal infection.[13,14] Neonates can acquire MRSA via vertical transmission[15] and breastfeeding,[16] and such colonization might affect the immune system development of neonates. According to a cohort study, infants born to mothers with nasal MRSA colonization are more likely to be colonized.[14] It has also been reported that nasal colonization of MRSA in pregnant women is a risk factor for surgical wound infection and may cause skin infections, mastitis, and other infections of pregnant women.[17,18] Because pregnant women undergoing cesarean delivery are at risk for wound infection, there may be some benefits to screening MRSA nasal colonization in this population, especially in the presence of other risk factors.[19] MRSA nasal colonization in pregnant women is hazardous to both neonates and pregnant women. Noticeably, compared with vertical and mammillary colonization of MRSA, nasal colonization with MRSA is essential to identify in pregnant women[20] and is easier to prevent and decolonize through effective precautions.

Therefore, to provide a scientific basis for the prevention and control of antibiotic-resistant *S aureus* infection in both neonates and pregnant women, we performed a cross-sectional study to determine the prevalence, influencing factors, and phenotypic and molecular characteristics of *S aureus* CC5 and MRSA CC5 nasal colonization in pregnant women in Shenzhen, China.

METHODS

Ethics statement

This study was approved by the Ethics Committee of Guangdong Pharmaceutical University and was performed in accordance with the Declaration of Helsinki. Prior to participation, all participants provided written informed consent.

Participants and questionnaires

A cross-sectional study was conducted between August and November 2015 in 2 hospitals in Shenzhen (Longhua Central Hospital and Guanlan People's Hospital), China. The participants were inpatients because of parturition. The following inclusion criteria were used: (1) pregnant Chinese women; (2) age between 35 and 40 weeks of gestation; (3) provision of signed informed consent; and (4) absence of acute diseases. A face-to-face questionnaire was then administered to collect demographic information and information regarding potential factors influencing *S aureus* colonization during pregnancy, such as age, education, average monthly income, frequency of handwashing, frequency of bathing, smoking, alcohol drinking, tea drinking, pet ownership, hours of computer use, hours of mobile phone use, family size (ie, number of people in the family, including the participant), and medical information.

Sample collection

While completing the questionnaires, the participants were sampled by trained personnel within 48 hours after being hospitalized. Two nasal swabs were collected from each participant (sterile cotton swabs were used in both anterior nares), and the swabs were stored in 5 mL of enrichment broth containing 1% tryptone, 7.5% NaCl, 1% mannitol, and 0.25% yeast extract. The broth tubes were immediately transferred to the laboratory at 4°C during transportation.

Isolation and identification

After 24 hours of incubation at 36°C ± 1°C, the swabs were then transferred to mannitol salt agar plates and incubated for 24-48 hours. Colonies that were yellow or had yellow zones on the mannitol salt agar plates were identified as *S aureus* if there were visible as grape-like clusters and gram-positive bacteria under a microscope and were positive for catalase, β-hemolysin, thermonuclease, and tube coagulase tests.[21,22] We isolated 1 isolate per sample.

Antimicrobial susceptibility testing

Antibiotic resistance testing was conducted using the Kirby-Bauer disk diffusion method following the Clinical and Laboratory Standards Institute guidelines.[23] All *S aureus* isolates underwent phenotype analysis for antibiotic resistance to 11 antimicrobial agents from the following 9 antibiotic classes: penicillins (cefoxitin 30 μg and penicillin 10 U), lincosamides (clindamycin 2 μg), ansamycins (rifampicin 5 μg), fluoroquinolones (moxifloxacin 5 μg), aminoglycosides (tobramycin 10 μg and gentamicin 10 μg), sulfonamides (sulfamethoxazole-trimethoprim 25 μg), oxazolidones (linezolid 30 μg), glycopeptides (teicoplanin 30 μg), and macrolides (erythromycin 15 μg). Interpretation of the zones of inhibition is reported as susceptible, resistant, or intermediately resistant (where applicable) according to the Clinical and Laboratory Standards Institute guidelines. In erythromycin-resistant isolates, inducible clindamycin resistance was assessed using the D-zone test.[24] *S aureus* isolates that were resistant to cefoxitin were identified as MRSA. Isolates that exhibited complete phenotypic resistance to at least 3 antibiotic classes were classified as multidrug resistant.[25] *S aureus* strain ATCC25923 (ATCC, Rockville, MD) was used for quality control.

Molecular characteristics

Molecular characteristics of isolates were identified through polymerase chain reaction assays. All *S aureus* isolates were tested for toxin genes (*PVL*, *TST*, *ETA*, and *ETB*).[26] Multilocus sequence typing for all *S aureus* isolates is a nucleotide sequence–based approach for 7 housekeeping genes, and it was previously performed using published primers and conditions for polymerase chain reaction assays.[27] Alleles and sequence types (STs) were determined using the multilocus sequence typing database, and singletons or members of a colonal complex (CC) were determined using the eBURST algorithm (accessible at http://eburst.mlst.net). All MRSA isolates underwent staphylococcal cassette chromosome *mec* (SCC*mec*) typing.[28]

Statistical analysis

All data were entered in duplicate into an EpiData (version 3.1) database (EpiData Association, Odense, Denmark). Variables were classified according to the median. We used log-binomial regression models to calculate the prevalence ratios and 95% confidence intervals comparing the nasal colonization prevalence for each *S aureus* and MRSA outcome in the CC5 groups with the non-CC5 and non–*S aureus* groups of pregnant women. We examined potential confounding covariates that were considered as potential confounders in adjusted log-binomial regression models. In addition, variables with a P value <.20 in univariate analysis (Pearson χ^2 test or Fisher exact test) were considered potential confounding covariates. We also conducted separate analyses to examine the phenotypic and molecular characteristics of *S aureus* isolates between CC5 and non-CC5 groups by using Pearson χ^2 test or Fisher exact test. A P value <.05 was considered statistically significant. All statistical analyses were 2-sided and conducted using Stata 13.1 (StataCorp, College Station, TX).