A prospective cohort pilot study of the clinical and molecular epidemiology of *Staphylococcus aureus* in pregnant women at the time of group B streptococcal screening in a large urban medical center in Chicago, IL USA

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Submitted: 06/04/13; Revised: 09/06/13; Accepted: 09/09/13

http://dx.doi.org/10.4161/viru.26435

**Keywords:** *Staphylococcus aureus*, epidemiology, pregnancy, molecular epidemiology, infections in obs and gyn

*Staphylococcus aureus* infects millions worldwide. Methicillin-sensitive and methicillin-resistant *S. aureus* (MSSA and MRSA) isolates may closely share virulence determinants through related clonal complexes. The purpose of this pilot study was to assess the epidemiology of *S. aureus* colonization in pregnant women in a community-acquired MRSA endemic area at the time of group B streptococcus screening. Of 107 women, 23 were colonized with MSSA, none with MRSA. Virulence factors Panton–Valentine leukocidin and ACME arcA were found in 75% and 6% of isolates, respectively. Mothers of infants with longer lengths of stay were 1.5 times more likely to be *S. aureus* colonized (*P = 0.07*). Postpartum infections occurred in 13%. The impact of colonization on maternal health should continue to be studied.

*Staphylococcus aureus* (*S. aureus*) is a common bacterial pathogen encountered by primary care providers and subspecialists alike. *S. aureus* frequently colonizes the anterior nares but also the skin, nails, pharynx, axilla, perineum, and vagina.¹ Reported rates of *S. aureus* vaginal colonization during pregnancy range from 4% to 22%.² ³ The overall prevalence of community-acquired MRSA (CA-MRSA) infection in Chicago, Illinois has increased over the last decade, with rates as high as 996 per 100,000 patients in certain high risk areas and patient populations.²

Recent data suggests that infants born to mothers who are *S. aureus* colonized are more likely to become colonized as neonates, but not necessarily infected, and this transmission is most often horizontal rather than vertical in nature.³ ⁴ However, colonized pregnant women, unlike their infants, are more likely to develop methicillin-sensitive or methicillin-resistant *S. aureus* (MSSA or MRSA) infections in the postpartum period than those who are not colonized.⁶

*S. aureus* is known to have remarkable pathogenic versatility, and MSSA strains have recently been shown to share clonal complex genetic lineages with CA-MRSA.⁷ Consequently, strains closely share virulence determinants such as exotoxins that comprise α-, β-, γ-, and δ-hemolysins, leukotoxins (such as Panton–Valentine leukocidin), exfoliative toxins, pyrogenic toxin superantigens, and enterotoxins.⁸ Other determinants known to contribute to the expression of virulence and/or enhanced bacterial fitness in MRSA such as the mobile elements arginine catabolic mobile element (ACME) arcA and staphylococcal chromosomal cassette mec (SCCmec) containing the antibiotic resistance mecA gene, and cell-wall-associated surface proteins (sasX), and capsular polysaccharides (capS) may be horizontally transferred into phylogenetically distinct MSSA.⁹ ¹² ACME arcA is an acquired genetic variant, distinct from chromosomal arcA (chrome arcA), a housekeeping gene which is commonly present in *S. aureus*.⁹ ACME arcA inserts into the orfX gene, adjacent to SCCmedVa cassette in the USA300 strain, a common CA-MRSA clone. It has also been detected in clonally related MSSA strains and likely enhances bacterial virulence and fitness.⁹

The purpose of our pilot study was to assess the clinical and molecular epidemiology of *S. aureus* nasal and vaginal colonization in a cohort of pregnant women in a CA-MRSA endemic area at the time of routine group B streptococcus (GBS) screening.

This study was a prospective cohort analysis of 107 women receiving prenatal care at two obstetrics clinics associated with Northwestern Memorial Hospital between February 2008 and June 2009. A total of 107 nasal and 107 vaginal swabs for *S. aureus* were obtained during the time of routine rectovaginal GBS screening between 35 and 37 weeks gestation. The two clinics (Prentice Ambulatory Clinic, Northwestern Memorial Faculty
De-identified anterior nares and vaginal swabs were sent to the Infectious Disease Laboratory of Ann and Robert H. Lurie Children’s Hospital of Chicago for culture examination. Swabs were plated within 24 h of collection on 5% blood agar plates (BBL TSA II Agar, Becton, Dickenson and Company) and incubated overnight at 36 °C. Plates were examined for evidence of staphylococcal colonies. Putative isolates were confirmed as S. aureus strains by coagulate testing. Strains were further tested for oxacillin resistance with Kirby Bauer susceptibility testing and confirmed as MRSA by PBP-II reactivity (Staphaurex PBP2a Latex Kit, Thermo Fisher Scientific Remel Products). All S. aureus isolates were sub-cultured for purity and frozen at −70 °C until required for additional testing.

Kirby Bauer antimicrobial susceptibilities were performed using standard CLSI methods and interpretive criteria.\(^13\)

Strain typing was done by PFGE as previously described using Smal as the restriction enzyme.\(^14,16\) The relatedness of isolates was based on visual comparison of bands and by generation of relatedness dendrograms using TotalLab TL120 software (TotalLab Ltd.).

S. aureus isolates were grown on 5% blood agar plates overnight. DNA was extracted using the QIAmp DNA Minikit (Qiagen, Inc.). PCR amplification was performed on all isolates for the presence of meca, arcA, ACME, sasX, lukSF-PV (PVL), and cap5 genes.\(^7,12,17,19\)

We analyzed a total of 214 isolates, 107 nasal and 107 vaginal, for the presence of S. aureus. Twenty-four (11%) isolates from 23 women (17 nasal, 7 vaginal) were found to have S. aureus, all MSSA. One woman exhibited both nasal and vaginal colonization. Charts were available for review in 102 women. Demographics of women colonized with S. aureus were similar to those not colonized with S. aureus (Table 1). We found that mothers with infants with longer lengths of stays were a little over 1.5 times more likely to be colonized with S. aureus (per each extra day of stay); however, this is only borderline statistical significance (\(P = 0.07\); OR = 1.635, 95% CI = 0.956, 2.794). Additionally, mothers having C-sections were found to be approximately one-third as likely to be colonized with S. aureus compared with mothers having other types of deliveries (\(P = 0.04\); OR = 0.31, 95% CI 0.096, 0.99). There appeared to be a trend toward multiparity being more common in S. aureus colonized mothers compared with non-colonized mothers (57% vs. 39%), but this was not statistically significant (\(P = 0.16\)).

No differences were found when comparing vaginal, nasal, and non-colonized when assessing race, marital status, age, zip code, county, type of insurance, obstetric clinic, GBS status, gravidity, receipt of antibiotics (prophylactic or therapeutic), or gestational age at the time of delivery. Data regarding postpartum follow-up in the S. aureus colonized was available for 15 women. Of these women, postpartum infection was identified in 2 (13%) within six weeks of delivery; however no cultures were taken. None of the infants born to colonized mothers were found to have infections during their hospitalization after birth. No additional or long-term follow-up was available.

Seventeen S. aureus isolates were available for further microbiologic and molecular testing. PFGE patterns revealed widely divergent stains by relationship analysis (Fig. 1) and corresponding variations in antibiograms (Table 2). Erythromycin resistance was present in 4 (25%) and levofloxacin resistance was found in 1 (6%) isolate. No strains were oxacillin (mexiteline) resistant. Sixteen isolates were available for toxin and virulence gene analysis (Table 2). Panton–Valentine leukocidin (PVL) was detected in 12 (75%) of MSSA isolates. Chrome arcA was detected in 12 (75%) of isolates, while the genetic variant ACME arcA was found in one strain (6%), which was a vaginal isolate. Other virulence and bacterial fitness markers, cap5 and sasX, were not detected in any MSSA isolates.

S. aureus is a common commensal bacterium with highly variable pathogenesis related to strain type. The emergence of CA-MRSA in part due to antibiotic selection pressure has led to the need for broader therapeutic regimens. In our region, CA-MRSA is common; however, in our patient population of pregnant women, we surprisingly found no CA-MRSA colonization. MSSA lineages once thought distinct from CA-MRSA now reveal close phylogenetic relationships between similar clonal complexes.\(^7\)
The staphylococcal methicillin resistance determinant, mecA, is part of a mobile genetic element, staphylococcus chromosomal cassette mec (SCCmec). Acquisition and retention of SCCmec is most likely in a restricted number of six permissive S. aureus clonal complexes that are determined by multilocus sequence typing of seven housekeeping genes. Not all circulating SCCmec clonal complexes are the classic SCCmecIVa associated with CA-MRSA. Other clonal complexes can retain mecA but at much lower frequency, and expression of methicillin resistance is low level and due to derepression of mecA gene transcription. This is consistent with our finding of only 1/16 (6%) tested MSSA isolates containing the mecA gene.

Chrome arcA is present in most S. aureus strains, is a regulatory gene related to anaerobic respiration transitions, and was identified in 75% of isolates. We additionally looked for the arginine catabolic mobile element (ACME) arcA, a mobile genetic element (which integrates into the same chromosomal site as SCCmec) related to virulence and bacterial fitness in MRSA. We found this present in only one isolate, however it suggests a possible genetic linkage and therefore potential expression of virulence factors thought more characteristic of certain MRSA lineages. We were somewhat surprised to find PVL in 75% of isolates. PVL is a known marker of virulence and has been associated increased severity of S. aureus infections, however its expression did not seem to impact the majority of our patients as only 13% of colonized women were known to develop postpartum infections. And although infants of mothers colonized with MSSA had longer lengths of stay, it did not appear to be due to early neonatal infections.

We recognize that our study has limitations. This was a small pilot study of a cohort of women at one tertiary care center, thus our results cannot be justifiably extrapolated to represent the general population; however, our population did represent a diverse group of mothers from two clinics.

The epidemiology of S. aureus continues to evolve. Strains detected as methicillin sensitive may share clonal complex genetic lineages with CA-MRSA and thus known virulence factors and enhanced bacterial fitness. The impact of colonization with such strains on maternal health, infection rates during pregnancy, and during the neonatal period should continue to be investigated.

Disclosure of Potential Conflicts of Interest
No conflicts of interest, financial, or other to report for any author.

Funding
Thrasher Research Fund New Researcher Award #NR-0038 (LKL); Children’s Memorial Research Fund (TQT).

Acknowledgments
We especially thank the nurse practitioners of Northwestern Memorial Hospital (NMH) MFM clinic Mary Ciszewski, Paula Kowalczuk, and Linda Jagieski for collection of samples and data. We would also like to thank Dr Dana Gossett, Sue Wathen, the NMH NMFF clinic, Ami Patel, Francesca Facco, and the resident physicians of NMH PAC clinic for all of their assistance with this project.

Table 1. Demographics characteristics of 102 pregnant women

| Characteristic | Women with S. aureus colonization n = 23 | Patients without S. aureus colonization n = 79 | P value |
|----------------|----------------------------------------|---------------------------------------------|---------|
| Nasal colonization* | 17                                      | NA                                          | NA      |
| Vaginal colonization | 7                                       | NA                                          | NA      |
| Age, mean years (SD) | 32.26 (5.01)                           | 32.94 (6.35)                                | 0.74    |
| # of maternal medical problems (SD) | 3.50 (3.59)                            | 3.58 (3.29)                                | 0.98    |
| Race/ethnicity | n (%)                                    |                                             |         |
| White/Caucasian/Other | 17 (74)                                 | 65 (82)                                     |         |
| Black/African-American | 3 (13)                                  | 4 (5)                                       |         |
| Hispanic/Latino | 3 (13)                                   | 10 (13)                                     |         |
| Marital status | n (%)                                    |                                             |         |
| Single | 7 (30)                                   | 20 (25)                                     |         |
| Married | 16 (70)                                  | 59 (75)                                     |         |
| Public Insurance | 3 (13)                                  | 13 (16)                                     | 0.99    |
| Multiparous | 16 (70)                                 | 49 (64)                                     | 0.8     |
| Multigravida | 13 (57)                                  | 31 (39)                                     | 0.16    |
| GBS positive | 3 (13)                                   | 12 (15)                                     | 0.83    |
| C-section | 4 (17)                                   | 32 (41)                                     | 0.04    |
| Antibiotics at delivery † | 7 (30)                                  | 39 (49)                                     | 0.11    |
| Gestational age, weeks (SD) | 38.97 (1.28)                            | 38.58 (1.58)                                | 0.21    |
| Infant length of stay, days (range) | 2.90 (1–7)                              | 2.26 (1–6)                                  | 0.07    |

*One woman was colonized both nasal and vaginally; †prophylactic or therapeutic.
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Figure 1. Relationship analysis of Staphylococcus aureus isolates.
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Table 2. Characteristics of Staphylococcus aureus in a cohort of pregnant women

| Isolate | Location | MecA | PVL | CapS | ACME ArcA | Chromo ArcA | SasX | OX | TMP/SMX | CC | ERY | LVX | LZD | RIF | Van |
|---------|----------|------|-----|------|-----------|-------------|------|----|---------|----|-----|-----|-----|-----|-----|
| 1       | N        | Neg  | Neg | Neg  | Neg       | Neg         | Neg  | S  | S       | S  | S   | R   | S   | S   | S   |
| 2       | N        | Neg  | Pos | Neg  | Neg       | Pos         | Neg  | S  | S       | S  | S   | S   | S   | S   | S   |
| 3       | N        | Neg  | Pos | Neg  | Neg       | Pos         | Neg  | S  | S       | S  | S   | R   | S   | S   | S   |
| 4       | N        | Neg  | Pos | Neg  | Neg       | Pos         | Neg  | S  | S       | S  | S   | S   | S   | S   | S   |
| 5       | N        | Neg  | Pos | Neg  | Neg       | Pos         | Neg  | S  | S       | S  | S   | S   | S   | S   | S   |
| 6       | N        | Neg  | Pos | Neg  | Neg       | Pos         | Neg  | S  | S       | S  | S   | S   | S   | S   | S   |
| 7       | N        | Neg  | Pos | Neg  | Neg       | Pos         | Neg  | S  | S       | S  | S   | R   | S   | S   | S   |
| 8       | N        | Neg  | Pos | Neg  | Neg       | Pos         | Neg  | S  | S       | S  | S   | S   | S   | S   | S   |
| 9       | N        | Neg  | Pos | Neg  | Neg       | Pos         | Neg  | S  | S       | S  | S   | S   | S   | S   | S   |
| 10      | N        | Neg  | Neg | Neg  | Neg       | Pos         | Neg  | S  | S       | S  | S   | R   | S   | S   | S   |
| 11      | N        | Neg  | Pos | Neg  | Neg       | Pos         | Neg  | S  | S       | S  | S   | S   | S   | S   | S   |
| 12      | N        | Neg  | Pos | Neg  | Neg       | Pos         | Neg  | S  | S       | S  | S   | S   | S   | S   | S   |
| 13      | V        | Neg  | Neg | Neg  | Pos       | Pos         | Neg  | S  | S       | S  | S   | S   | S   | S   | S   |
| 14      | V        | Neg  | Pos | Neg  | Neg       | Neg         | Neg  | S  | S       | S  | S   | S   | S   | S   | S   |
| 15      | V        | Neg  | Pos | Neg  | Neg       | Pos         | Neg  | S  | S       | S  | S   | S   | S   | S   | S   |
| 16      | V        | Neg  | Neg | Neg  | Neg       | Neg         | Neg  | S  | S       | S  | S   | R   | S   | S   | S   |
| 17      | V        | Pos  | Pos | Neg  | Neg       | Neg         | Neg  | S  | S       | S  | S   | S   | S   | S   | S   |

N, nasal; V, vaginal; Pos, positive; Neg, negative; PVL, Panton–Valentine leukocidin; ACME, arginine catabolic mobile element; Ox, oxacillin; TMP/SMX, trimethoprim/sulfamethoxazole; CC, clindamycin; Ery, erythromycin; LVX, levofloxacin; LZD, linezolid; RIF, rifampin; Van, vancomycin.