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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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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 *arc*A 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%.[2-4] 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.[5]

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.[3,6] 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.[7] 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) *arc*A and staphylococcal chromosomal cassette mec (SCC*mec*) containing the antibiotic resistance *mec*A gene, and cell-wall-associated surface proteins (sasX), and capsular polysaccharides (cap5) may be horizontally transferred into phylogenetically distinct MSSA.[8-12] ACME *arc*A is an acquired genetic variant, distinct from chromosomal *arc*A (chrome *arc*A), a housekeeping gene which is commonly present in *S. aureus*.[9] ACME *arc*A inserts into the *orfX* gene, adjacent to SCC*mec*IVa 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

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Foundation [NMFF], including those followed by the Maternal Fetal Medicine service) comprise a diverse group of women of all ages and from various socioeconomic and ethnic backgrounds. The protocol was approved by the Institutional Review Boards of Northwestern University and Ann and Robert H. Lurie Children's Hospital of Chicago. All women underwent informed consent prior to inclusion in the study for mother and infant.

Charts of mothers colonized with *S. aureus* were reviewed for demographic data, past medical and surgical history, risk factors for colonization such as exposure to health care facilities, for infectious conditions during their postpartum hospitalization and follow-up and compared with those who were not colonized. Charts of infants born to colonized mothers were also reviewed for early infectious processes during their admission after delivery.

Maternal and infant history variables were examined as possible predictors of three outcomes: (1) mother's *S. aureus* status (nasal or vaginal); (2) vaginal *S. aureus* as compared with uncolonized; and (3) vaginal *S. aureus* status vs. nasal, among colonized mothers. Predictors examined include age of mother, gestational age, race-ethnicity, GBS, gravida, antibiotics, insurance status, length of stay of the mother and infant, number of maternal medical problems, number of other living children, and other variables.

For dichotomous predictor variables (marital status, public vs. private insurance, antibiotics during pregnancy or at delivery, primigravida vs. multigravida, C-section delivery), Chi-square (or the Fisher exact test when cell sizes were small) were used to test for a statistically significance association with any of the three outcome variables. For continuous variables (mother's age, gestational age, length of stay) and categorical variables with more than two possible values (race/ethnicity, GBS, county of residence), logistic regression was used to determine statistical significance of the association with the three outcomes. All statistical analyses were performed using Statistical Analysis Software version 9.1.

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 coagulase 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 SmaI 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 *mec*A, *arc*A, ACME, *sas*X, *luk*SF-PV (PVL), and *cap*5 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* as 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, gravida, 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 (methicillin) 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 *arc*A was detected in 12 (75%) of isolates, while the genetic variant ACME *arc*A was found in one strain (6%), which was a vaginal isolate. Other virulence and bacterial fitness markers, *cap*5 and *sas*X, 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]

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* (%)**		**0.43**
White/Caucasian/Other	17 (74)	65 (82)	
Black/African-American	3 (13)	4 (5)	
Hispanic/Latino	3 (13)	10 (13)	
Marital status	***n* (%)**		**0.62**
Single	7 (30)	20 (25)	
Married	16 (70)	59 (75)	
Public Insurance	3 (13)	13 (16)	0.99
Multigravida	16 (70)	49 (64)	0.8
Multiparity	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.

The staphylococcal methicillin resistance determinant, *mec*A, is part of a mobile genetic element, staphylococcus chromosomal cassette *mec* (SCC*mec*). Acquisition and retention of SCC*mec* 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.[17] Not all circulating SCC*mec* clonal complexes are the classic SCC*mec*IVa associated with CA-MRSA. Other clonal complexes can retain *mec*A but at much lower frequency, and expression of methicillin resistance is low level and due to derepression of *mec*A gene transcription.[17,20,21] This is consistent with our finding of only 1/16 (6%) tested MSSA isolates containing the *mec*A gene.

Chrome *arc*A is present in most *S. aureus* strains, is a regulatory gene related to anaerobic respiration transitions,[9] and was identified in 75% of isolates. We additionally looked for the arginine catabolic mobile element (ACME) *arc*A, a mobile genetic element (which integrates into the same chromosomal site as SCC*mec*) related to virulence and bacterial fitness in MRSA.[9] 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,[7] 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.

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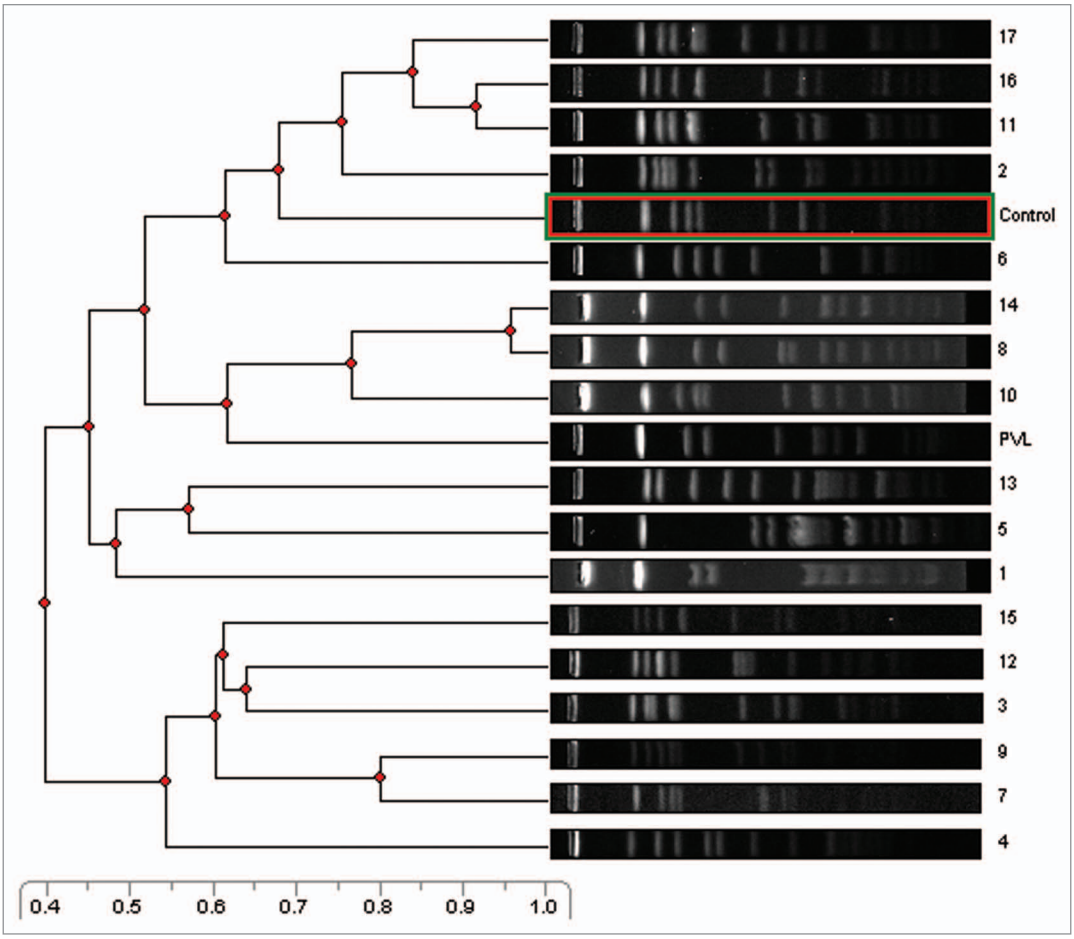

Figure 1. Relationship analysis of *Staphylococcus aureus* isolates.

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Table 2. Characteristics of *Staphylococcus aureus* in a cohort of pregnant women

Isolate	Location	Toxin/virulence gene profile						Kirby Bauer disc assays							
		MecA	PVL	Cap5	ACME ArcA	Chrome 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	Neg	Neg	Neg	Pos	Neg	S	S	S	R	S	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	Neg	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	R	S	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	R	S	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	R	S	S	S	S
17	V	Pos	Pos	Neg	Neg	Pos	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.

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