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Abstract

Colonization and infection by resistant strains of *Staphylococcus aureus* are being reported in epidemic proportions. The goal of this study was to determine the local prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in pregnant women in southern Nevada and how it correlates with colonization and infection of their neonates. Signed consent was obtained, and a brief questionnaire was administered by the medical staff to each pregnant woman to collect demographic data and pertinent medical, family and social history. Nasal and vaginal specimens were obtained from pregnant women at ≥35 weeks gestation, and nasal and umbilicus specimens were obtained from their newborns. Specimens were cultured onto two selective media for *S. aureus* and MRSA. Potential MRSA isolates were further evaluated for susceptibility to antibiotics. Specimens from 307 pregnant women and 174 neonates were collected, resulting in 172 mother-neonate paired specimens. A total of 278 questionnaires were received from study participants. MRSA prevalence in pregnant women was 1.0% and 0.3% for nasal and vaginal specimens, respectively. The MRSA prevalence in neonates was 0% and 0.6% for nasal and umbilical specimens, respectively. Four different antimicrobial susceptibility profiles were observed among the MRSA isolates. The results did not show transmission of MRSA from pregnant women to their newborns, or infections of newborns with MRSA. It is expected that the results of this study will inform future decisions on surveillance, treatment and prevention of MRSA infections in Nevada.

Key words antimicrobial susceptibility, colonization, methicillin-resistant *Staphylococcus aureus*, MRSA

Introduction

Colonization and infection by community-associated resistant strains of *Staphylococcus aureus* are being reported in epidemic proportions in many areas of the United States and around the world (Creech II, Kernodle, Alsentzer, Wilson, & Edwards, 2005; Faria et al., 2005; Mulvey et al., 2005; Taiwo et al., 2005). Although more frequently associated with skin and soft tissue infections, community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has been implicated in invasive infections in children, with wide geographical diversity in rates of colonization and infection (Kaplan et al., 2005; Ochoa, Mohr, Wanger, Murphy, & Heresi, 2005; Purcell & Fergie, 2005). Studies have shown that MRSA is a common bacterium in the Neonatal Intensive Care Unit (NICU) (Chen, Huard, Della-Latta, & Saiman, 2006; Khoury, Jones, Grim, Dunne, & Fraser, 2005). MRSA is recognized as a cause of infection among newborns in the NICU, and the existence of MRSA colonization in pregnant women has potential health implications for their neonates (Beigi & Hanrahan, 2007). A recent study identified MRSA colonization in prenatal vaginal cultures and raised questions with regards to pregnant women and their infants' wellbeing (Chen, Huard, Della-Latta, & Saiman, 2006). This may be due to the fact that persons colonized with MRSA are usually at a higher risk for infections and possibly transmit the bacterium to other family members (Chen, Huard, Della-Latta, & Saiman, 2006). Various studies have addressed the problem of MRSA in the NICU (Chen, Huard, Della-Latta, & Saiman, 2006; Chuang et al., 2004; Khoury, Jones, Grim, Dunne, & Fraser, 2005),

but there are limited data regarding the relationship between maternal vaginal colonization and the rate of infection found in their neonates. In addition, no consensus guidelines exist regarding the appropriate evaluation and treatment of previously healthy term and late-preterm neonates with community-associated *S. aureus* infections, especially those caused by CA-MRSA isolates (Fortunov, Hulten, Hammerman, Mason, & Kaplan, 2007).

The increasing rate of MRSA cases has led many clinicians to utilize newer staphylococcal antimicrobial agents as first-line empiric therapy. While some studies report equal efficacy or superiority of linezolid to vancomycin in treatment of MRSA (Sharpe, Shively, & Polk Jr., 2005; Shorr, Kunkel, & Kollef, 2005), there are reports of increasing clindamycin and rifampicin resistance among MRSA and of unstable vancomycin heteroresistance among clinical isolates of MRSA (Braun, Craft, Williams, Tuamokumo, & Ottolini, 2005; Plipat, Livni, Bertram, & Thompson, 2005; Tosun et al., 2005). The two most important considerations in choosing empiric antibiotic therapy are the knowledge of the most likely pathogen and the most likely active antimicrobial agent. With the increasing prevalence of MRSA, the large geographical diversity in colonization and infection rates, and the presence of increasing resistance to available therapies, clinicians should be aware of their local resistance rates. One method of gaining this knowledge is to maintain surveillance programs (both inpatient and outpatient) in order to identify local colonization rates and their relationship to clinical infections, as well as antibiotic resistance patterns. The goal of this study was to determine the prevalence of MRSA colonization in pregnant women and its potential association with MRSA colonization and infection in newborns delivered at the University Medical Center (UMC) in Las Vegas, Nevada.

Materials and Methods

Study Procedures

Nares and vaginal swabs were collected from 307 pregnant women at ≥35 weeks gestation presenting for care or delivery at three sites: Women's Center, Patient Care Center, and Birthing Center of the University Medical Center in Las Vegas, Nevada. Signed consent was obtained, and a brief questionnaire was administered by the medical staff to each pregnant woman to collect demographic data and pertinent medical, family and social history. Nares and umbilical swabs were collected from 174 neonates prior to discharge from the hospital. Specimens were cultured onto two selective media for the isolation of *S. aureus* and MRSA. The VITEK® 2 Compact (bioMérieux, Durham, NC) and the Kirby-Bauer disc diffusion method were used to characterize isolates based on their susceptibility to antimicrobial agents. Data were analyzed statistically to determine MRSA prevalence rates and to provide descriptive summary statistics across all collected variables for each study participant.

Test Organisms and Culture Media

Seven bacterial reference strains obtained from the American Type Culture Collection (Manassas, VA) were used in this study: *Escherichia coli* ATCC 25922, *S. aureus* ATCC 29213 and ATCC 25923 (methicillin-sensitive strains), *S. aureus* ATCC 43300 (a MRSA strain), *S. epidermidis* ATCC 12228, *S. saprophyticus* ATCC 15305, and *Proteus mirabilis* ATCC 12453. All media were obtained from BD Diagnostics, Sparks, MD, and consisted of BBL™ CHROMagar™ Staph aureus, BBL™ CHROMagar™ MRSA, BBL™ Oxacillin Screen Agar (OSA), BBL™ Mueller Hinton II agar, Difco™ Tryptic Soy Agar (TSA), and Trypticase™ Soy Agar with 5% Sheep Blood (TSAB). All cultures were incubated at 35°C for 24–48 hours.

Specimen Collection and Processing

Nares specimens were collected by medical staff with a dry, sterile swab (BBL CultureSwab; BD Diagnostics) which was inserted into each nostril of each subject, rotated for 5 seconds, and placed into a tube of liquid Stuart transport medium (BD Diagnostic). Vaginal specimens were collected at the same time specimens were collected for Group B streptococcus screening by swabbing the vaginal vault using a dry, sterile swab. Umbilical specimens were collected 24 hours after birth and prior to discharge from the hospital by swabbing the umbilical stump. Specimens were refrigerated, transported to the laboratory within 48 hours, and processed immediately upon arrival. Nares specimens were streaked for isolation onto BBL CHROMagar™ Staph aureus and BBL CHROMagar™ MRSA; all agar plate media were incubated in ambient atmosphere at 35°C for 24 hours (Figure 1). Media performance characteristics were obtained from the manufacturer's package inserts. Mauve to orange/mauve colonies produced on CHROMagar™ Staph aureus medium were identified as *S. aureus* isolates. Smooth, moderately sized mauve colonies which appeared on CHROMagar™ MRSA medium at 24 hours were interpreted as MRSA isolates. CHROMagar™ MRSA plates without typical MRSA colonies were incubated for an additional 24 hours. Mauve colonies appearing at 48 hours and those with atypical growth

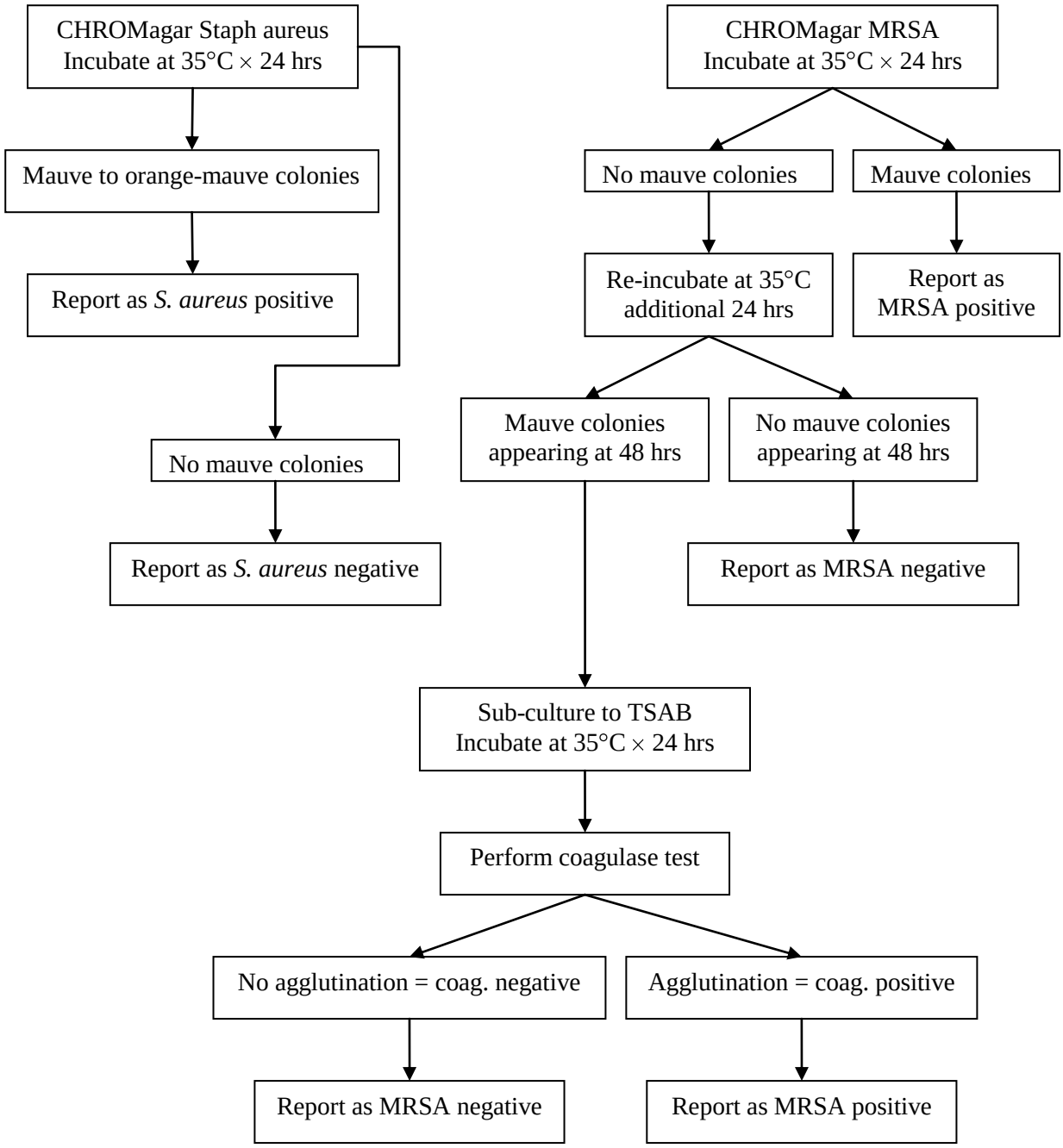

Figure 1 Flow chart illustrating the experimental design for nares sites using CHROMagar Staph aureus and CHROMagar MRSA (TSAB = 5% sheep blood agar; coag. = coagulase).

rates or colony morphology were subcultured to TSAB, incubated in ambient atmosphere at 35°C for 24 hours, then tested for coagulase activity (ASI Staphslide Latex Test; Arlington Scientific, Inc., Springville, UT) according to the manufacturer's instructions.

Umbilical and vaginal specimens were streaked for isolation onto CHROMagar™ Staph aureus and incubated as previously described. *S. aureus* isolates

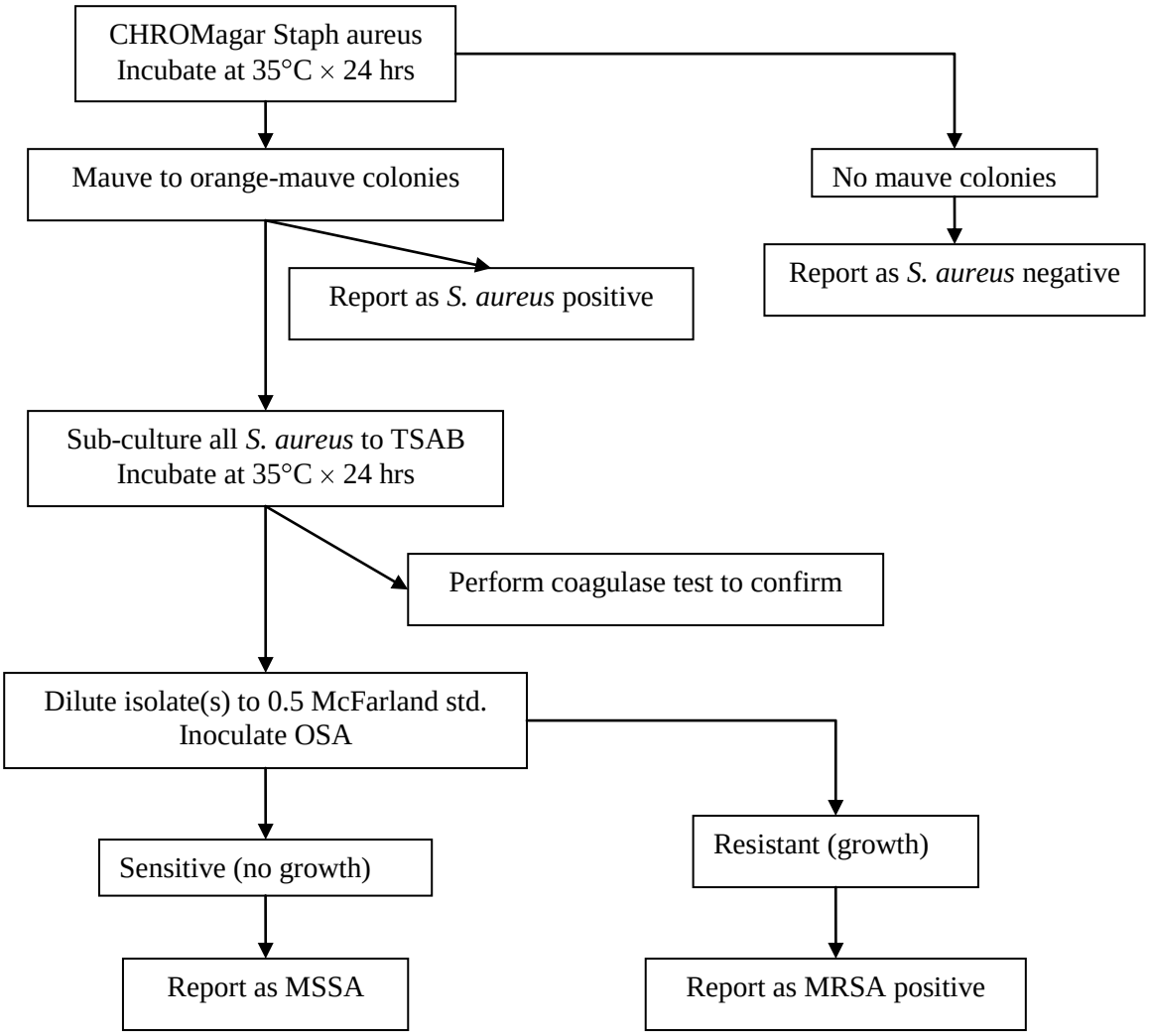

Figure 2 Flow chart illustrating the experimental design for sites other than nares using CHROMagar Staph aureus (TSAB = 5% sheep blood agar; OSA = Oxacillin Screen Agar; MSSA = methicillin sensitive *Staphylococcus aureus*).

were sub-cultured onto TSAB and incubated in ambient atmosphere at 35°C for 24 hours. The resulting colonies were tested for oxacillin susceptibility using OSA following the manufacturer's guidelines (Figure 2). All presumptive MRSA isolates were subcultured onto TSAB, tested for coagulase activity, and were stored at –70°C.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed with the VITEK® 2 Compact System (bioMérieux, Durham, NC) using the AST-GP67 card; antimicrobials and concentrations are shown in Table 1.

MRSA isolates and control culture *S. aureus* ATCC 25923 were subcultured onto TSA and incubated for 18-24 hours in ambient atmosphere at 35°C. A suspension of each isolate, at an optical density of 0.5-0.63 McFarland standard, was loaded onto an AST-GP67 card and processed in the VITEK 2 Compact according to the manufacturer's protocols. Susceptibility profiles were stored electronically in the system and evaluated. For comparison purposes, isolates were also tested using the Kirby-Bauer disc diffusion method on Mueller Hinton medium according to Clinical and Laboratory Standards Institute (CLSI; Wayne, PA) standards; antimicrobials and concentrations are shown in Table 1. All antimicrobial agents were obtained from

Table 1 Agents used in antimicrobial susceptibility testing of MRSA isolates (N/A = not applicable; CC or CM = Clindamycin; E = Erythromycin).

VITEK® 2 Compact		Disc Diffusion Method	
Antimicrobial Agent	**Concentration (μg/ml)**	**Antimicrobial Agent**	**Concentration (μg)**
Benzylpenicillin	0.125, 0.25, 1, 2, 8, 64		
Beta-lactamase	N/A		
Cefoxitin screen	6		
Ciprofloxacin	1, 2, 4		
Clindamycin	0.5, 1, 2	Clindamycin	2
Erythromycin	0.25, 0.5, 2	Erythromycin	15
Gentamicin	8, 16, 64	Gentamicin	10
Inducible Clindamycin resistance	CM 0.5; CM/E 0.25/0.5	Inducible Clindamycin resistance (CC/E)	2/15
Levofloxacin	0.25, 2, 8	Levofloxacin	5
Linezolid	0.5, 1, 2	Linezolid	30
Moxifloxacin	0.25, 2, 8		
Nitrofurantoin	16, 32, 64		
Oxacillin	0.5, 1, 2		
Quinupristin/Dalfopristin	0.25, 0.5, 2		
Rifampicin	0.25, 0.5, 2	Rifampin	5
Tetracycline	0.5, 1, 2	Tetracycline	30
Tigecycline	0.25, 0.5, 1		
Trimethoprim/ Sulfamethoxazole	2/38, 8/152, 16/304	Trimethoprim/ Sulfamethoxazole	1.25/23.75
Vancomycin	1, 2, 4, 8, 16	Vancomycin	30

Becton Dickinson. Organisms included in testing for quality control purposes were *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. Presumptive MRSA isolates were subcultured onto TSA and incubated for 24 hours as described above. Each bacterial suspension was prepared according to the antimicrobial agent manufacturer's instructions by inoculating 5 ml of Bacto™ Tryptic Soy Broth (BD Diagnostics) with a few isolated colonies and the suspension diluted as needed to obtain turbidity equivalent to a 0.5 McFarland standard. Within 15 minutes of preparation, a sterile cotton swab was dipped into the suspension; excess liquid was expressed from the swab. The agar surface was inoculated three times, rotating the plate 60° each time, then sweeping the swab around the outer rim of the agar. The plates were allowed to dry at room temperature for 3–5 minutes before applying the discs. Four and five discs, respectively, were placed manually onto each plate with sterile forceps and pressed firmly onto the surface. To detect inducible clindamycin resistance, double disc diffusion testing (D test) was performed by placing the clindamycin and erythromycin discs 15 mm apart (center to center); all other discs were placed 30 mm apart. Plates were incubated aerobically at 35°C and zone diameters were measured after 16-18 hours of incubation; oxacillin disc zone diameters were measured at 24 hours. Zone diameters were measured to the nearest millimeter and results were recorded as susceptible, intermediate, or resistant based on interpretive criteria provided by BD Diagnostics.

Strain Typing

Strain typing of MRSA isolates was performed using the DiversiLab® System (bioMérieux, Inc.) according to manufacturer's instructions. Briefly, a 10 µl

loopful of cells was harvested from a 24 hour culture of each isolate grown on TSAB, and the DNA was extracted using the UltraClean™ Microbial DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA). Genomic DNA concentration was measured spectrophotometrically and normalized prior to performing repetitive polymerase chain reaction (rep-PCR) amplification. Rep-PCR amplification was performed with the DiversiLab® *Staphylococcus* Fingerprinting Kit in a GeneAmp® PCR System 2400 (Applied Biosystems, Foster City, CA) following the DiversiLab® protocol. The PCR product was loaded onto the DiversiLab® LabChip and analyzed according to the manufacturer's instructions. Strain profiles were compared to isolates in the bioMérieux database with the DiversiLab® software. Isolates were grouped according to the closest match from the bioMérieux database's isolates showing 90% similarity. Additional review of the virtual gel images, graph overlays and similarity matrix were performed for samples showing similarities to two different strain types.

Statistical Methods and Analyses

Based on a yearly total of 5,000 births at UMC and a 5% margin of error, it was estimated through power analysis that 360 participants would be needed to provide a population MRSA colonization prevalence rate with 95% confidence. Prevalence of MRSA and *S. aureus* colonization were determined for mothers and newborns, along with strain type and antimicrobial susceptibility data. Further, descriptive statistics for both quantitative and qualitative variables were calculated and summarized. All statistics were calculated using SPSS version 17.0, SAS version 9.2, and NCSS/PASS 2004.

Results

Specimen Collection and Analysis

Specimens from a total of 307 pregnant women (i.e., 307 vaginal specimens and 307 nasal specimens) and 174 neonates (i.e., 174 umbilical specimens and 174 nasal specimens) were collected. From these specimens, a total of 172 mother-neonate pairs were obtained. The prevalence of MRSA in pregnant women was 1.0% and 0.3% for nares and vaginal specimens, respectively. In neonates, MRSA colonization prevalence was 0% and 0.6% for nares and umbilicus specimens, respectively. The prevalence of methicillin susceptible *Staphylococcus aureus* (MSSA) in pregnant women was 16.0% and 5.2% for nares and vaginal specimens, respectively. The prevalence of MSSA in neonates was 0.6% and 1.7% for nares and umbilicus specimens, respectively.

Antimicrobial Susceptibility Testing

Antimicrobial agent susceptibility results obtained with the VITEK 2 Compact for MRSA positive specimens are shown in Table 2. Three different susceptibility profiles were obtained among the five MRSA positive isolates. All five isolates were resistant to benzylpenicillin and oxacillin, four were resistant to erythromycin, and three isolates were resistant to ciprofloxacin and showed intermediate resistance to levofloxacin.

Antimicrobial agent susceptibility results obtained with the Kirby-Bauer disc diffusion method for MRSA positive specimens confirmed data obtained with the VITEK 2 Compact, with the exception that isolates showing intermediate levofloxacin resistance with the VITEK 2 Compact were determined to be resistant with the Kirby-Bauer method (Table 3).

Strain Typing

No identical matches were obtained between the MRSA isolates in this study and the MRSA strains in the bioMérieux DiversiLab® System database. Three isolates with identical antimicrobial agent susceptibility profiles all were identified as being most closely related to the Iberian MRSA strain (95.4% to 97.6% similarity). However, similarity to Brazilian (94.6% to 97.0%) and USA 700 (95.3% to 95.8%) strains was also observed (data not shown).

Questionnaire Data

A total of 278 questionnaires were received with the 307 specimens from pregnant women. The number of respondents between categories varied due to incomplete or missing information obtained for some questions. Basic summary statistics from the questionnaire responses from pregnant women showed a median age of 26 years of age (N = 243; Table 4).

The majority of the pregnant women that participated in the study were of Hispanic ethnicity (80.3%), followed by Caucasian (8.4%) and African American (7.3%; N=274; Table 4). The medical history of pregnant women revealed that 18 of 278 (6.5%) had *Chlamydia* and 28 of 278 (10.1%) had an abnormal PAP smear result in the past year (Table 5). Three participants reported a previous *Staphylococcus* infection and were treated with antibiotics.

Analysis of the questionnaire data from participants colonized by MRSA showed that the age of pregnant women ranged between 22.5 and 34.3, and that three were of Hispanic ethnicity and one was African American (Table 6). Potential risk factors documented for these pregnant women included

Table 2 Antimicrobial agent susceptibility results obtained with the VITEK 2 Compact for MRSA positive specimens. *S. aureus* ATCC 25923 was used as QC organism. Antimicrobial agent susceptibility (Minimum Inhibitory Concentration) was determined with the CLSI M100-S16 (2006) Interpretation Guidelines (S = susceptible; I = intermediate; R = resistant; + represents positive; - represents negative).

Antimicrobial Agent	**Conc. (μg/ml)**	**MRSA Positive Specimen**				
		73MN	207MV	241MN	248MN	377BU
Benzylpenicillin	0.125, 0.25, 1, 2, 8, 64	**R**	**R**	**R**	**R**	**R**
Beta-lactamase	N/A	+	+	+	+	+
Cefoxitin screen	6	+	+	+	+	+
Ciprofloxacin	1, 2, 4	**R**	**R**	S	S	**R**
Clindamycin	0.5, 1, 2	S	S	S	S	S
Erythromycin	0.25, 0.5, 2	**R**	**R**	S	**R**	**R**
Gentamicin	8, 16, 64	S	S	S	S	S
Inducible Clindamycin resistance	CM 0.5; CM/E 0.25/0.5	-	-	-	-	-
Levofloxacin	0.25, 2, 8	I	I	S	S	I
Linezolid	0.5, 1, 2	S	S	S	S	S
Moxifloxacin	0.25, 2, 8	S	S	S	S	S
Nitrofurantoin	16, 32, 64	S	S	S	S	S
Oxacillin	0.5, 1, 2	**R**	**R**	**R**	**R**	**R**
Quinupristin/Dalfopristin	0.25, 0.5, 2	S	S	S	S	S
Rifampicin	0.25, 0.5, 2	S	S	S	S	S
Tetracycline	0.5, 1, 2	S	S	S	S	S
Tigecycline	0.25, 0.5, 1	S	S	S	S	S
Trimethoprim/ Sulfamethoxazole	2/38, 8/152, 16/304	S	S	S	S	S
Vancomycin	1, 2, 4, 8, 16	S	S	S	S	S

Table 3 Antimicrobial agent susceptibility results obtained with the Kirby-Bauer disc diffusion method for MRSA positive specimens. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as QC organisms. Antimicrobial agent susceptibility was determined with the Zone Diameter for *Staphylococcus* spp. Interpretive Standards (S = susceptible; R = resistant; - represents negative result; CC = Clindamycin; E = Erythromycin).

Antimicrobial Agent	**Conc. (μg)**	**MRSA Positive Specimen**				
		73MN	207MV	241MN	248MN	377BU
Clindamycin	2	S	S	S	S	S
Erythromycin	15	**R**	**R**	S	**R**	**R**
Gentamicin	10	S	S	S	S	S
Inducible Clindamycin resistance (CC/E)	2/15	-	-	-	-	-
Levofloxacin	5	**R**	**R**	S	S	**R**
Linezolid	30	S	S	S	S	S
Rifampin	5	S	S	S	S	S
Tetracycline	30	S	S	S	S	S
Trimethoprim/Sulfamethoxazole	1.25/23.75	S	S	S	S	S
Vancomycin	30	S	S	S	S	S

Table 4 Demographics from pregnant women and their neonates born at University Medical Center (S.D. = standard deviation).

Mother's Age (yrs)				
N	**Mean (yrs.)**	**S.D.**	**Minimum**	**Maximum**
243	26.0	6.18	14.8	42.0
Race/Ethnicity of Mother (N = 274)				
Race		**Frequency**		**Percent**
Hispanic		220		80.3
Caucasian		23		8.4
African American		20		7.3
Other		6		2.2
Native American		3		1.1
Asian		1		0.4
Middle Eastern		1		0.4
Number of Pregnancies (N = 276)				
Number		**Frequency**		**Percent**
1		97		35.1
2		72		26.1
3		45		16.3
4		27		9.8
5		12		4.3
6		8		2.9
7		3		1.1
8		9		3.3
9		1		0.4
12		1		0.4
16		1		0.4
Number of Previous Live Births (N = 239)				
Number		**Frequency**		**Percent**
0		72		30.1
1		68		28.5
2		50		20.9
3		23		9.6
4		10		4.2
5		10		4.2
6		3		1.3
7		1		0.4
10		1		0.4
11		1		0.4
Neonate Gender (N = 225)				
Gender		**Frequency**		**Percent**
Female		106		47.1
Male		119		52.9

Table 5 Medical history from pregnant women (PAP = Papanicolaou test; STD = sexually-transmitted disease).

Previous *Staphylococcus* Infection (N = 278)				
Participant ID	**Year**	**Site**	**Treatment**	**Patient History**
99	2008	Abdomen	Antibiotics	• Hashimoto's disease • Surgery in 2006 (cleft lip) • Imprisonment in the last year • Antibiotic taken in last 6 months • Nose/skin/throat problems • Abnormal PAP in last year
277	1999	Arm	Antibiotics	• Has had a family member with kidney failure requiring dialysis • Antibiotic taken in the last 6 months (self and other household member) • *Chlamydia* in the last year • Abnormal PAP in the past
278	1996	Unreported	Antibiotics	• Surgery in 1986 (lymphadenectomy) • Surgery in 1998 (C-section) • Family member with chronic skin disease • Reports working with patients in hospital • Circulation problems • Antibiotic taken in the last 6 months

Responses to Having STDs/Abnormal PAP smear (N = 278)				
STD	**Overall**		**Past Year**	
	Frequency Reporting Positive	**Percent Reporting Positive**	**Frequency Reporting Positive**	**Percent Reporting Positive**
Syphilis	1	0.4	0	0.0
Chlamydia	31	11.2	18	6.5
Gonorrhea	5	1.8	2	0.7
Abnormal PAP	39	14.0	28	10.1

STDs/Abnormal PAP in Medical Record (N = 278)		
STD	**Frequency Reporting Positive**	**Percent Reporting Positive**
Syphilis	0	0.0
Chlamydia	6	2.2
Gonorrhea	2	0.7
Abnormal PAP	7	2.5

Comparison of medical record to self report of STDs/Abnormal PAP (N=278)

- Syphilis was in medical record 0 times, and reported 1 time
- Gonorrhea was in medical record 2 times and reported 5 times
- Chlamydia was in medical record 6 times and reported 31 times
- Abnormal PAPs were in medical record 7 times but were reported 39 times

Table 6 Demographics and characteristics of participants with MRSA positive specimens (Unknown = information not provided; N/A = not applicable; N/D = not determined; N = 278).

Participant ID	Who was colonized?	Age (yrs.)	Ethnicity	Was neonate also colonized if mother positive?	Where was the organism isolated?	Potential risk factors or medical history
73	Mother	34.3	Hispanic	No	Nares	• Urinary tract, bladder or kidney infections in the last year • Antibiotic use in the last 6 months
207	Mother	25.3	Hispanic	N/D	Vaginal	• C-section delivery with this pregnancy
241	Mother	Unknown	African American	No	Nares	• Antibiotic use by household member in the last 6 months
248	Mother	22.5	Hispanic	No	Nares	• Hypertension • Household member hospitalized in the last year • Family history of skin disease • Abnormal PAP in the last year
377	Neonate	Newborn	Unknown	N/A	Umbilicus	• Mother reports urinary tract, bladder or kidney infections in the last year

urinary tract, bladder or kidney infections in the previous year, antibiotic use in the previous six months by the participant or a household member, hypertension, hospitalization of household member in the previous six months, family history of skin disease, and abnormal PAP smear result in the previous year (Table 6).

Discussion

Previous studies of MRSA colonization in pregnant women have shown nasal carriage rates ranging from 1.3 to 2.1%, and vaginal carriage rates ranging from 0.3 to 1.0% (Beigi & Hanrahan, 2007; James et al., 2008; Mitsuda, Arai, Fujita, & Yokota, 1996; Reusch et al., 2008). The MRSA colonization prevalence of 1.0% and 0.3% for nares and vaginal specimens, respectively, measured for pregnant women in this study is within the range previously observed. Studies of MRSA colonization in neonates have indicated nasal carriage rates ranging from 0.3 to 3.9%, and an umbilical carriage rate of 0.7% (James et al., 2008; Mitsuda, Arai, Fujita, & Yokota, 1996; Reusch et al., 2008). The MRSA colonization prevalence of 0% and 0.6% for nares and umbilicus specimens, respectively, measured for neonates in this study is consistent with data obtained in a survey conducted in 2005–2006 (Reusch et al., 2008). Mitsuda *et al*. obtained a higher MRSA prevalence rate in nares (i.e., 3.9%); however, these specimens were collected from 6-day-old neonates (Mitsuda, Arai, Fujita, & Yokota, 1996) and in the present study neonatal specimens were collected at age 1 to 2-days-old. Five MRSA positive specimens were obtained in this study, and consisted of four from pregnant women (i.e., 3 nares, 1 vaginal) and one from a neonate (i.e., umbilicus). While these data did not show transmission between MRSA positive pregnant women and their newborns, it has been suggested that newborn colonization becomes apparent 2-3 days or more after birth (Cimolai, 2003). Previous studies have demonstrated increased MSSA nasal colonization rates over time, for newborns, from 3.1% at 1-day-old to 21.9% at 3-days-old (Cvetniæ, Kuèišec-Tepeš, Šeper, & Šips, 1991). Documented MSSA transmission rates between pregnant women and their newborns have been very low, and vertical transmission of MRSA may be <1% (Mitsuda, Arai, Fujita, & Yokota, 1996). In some studies, MRSA transmission from pregnant women to newborns delivered vaginally was not detected (Mitsuda, Arai, Fujita, & Yokota, 1996; Reusch et al., 2008).

In pregnant women, MSSA was present in 16.0% of the nasal specimens, within the estimates of 6.9 and 23.0% obtained in previous studies (Beigi & Hanrahan, 2007; James et al., 2008; Mitsuda, Arai, Fujita, & Yokota, 1996). Similarly, the MSSA vaginal prevalence of 5.2% obtained in this study was within the rates of 1.0 and 7.5% previously observed (Beigi & Hanrahan, 2007; James et al., 2008; Mitsuda, Arai, Fujita, & Yokota, 1996). In neonates, the MSSA colonization prevalence of 0.6% for nares specimens was below the range of 3.1 and 10.1% obtained in previous surveys (Cvetniæ, Kuèišec-Tepeš, Šeper, & Šips, 1991; James et al., 2008; Mitsuda, Arai, Fujita, & Yokota, 1996). The higher prevalence rate of 10.1% MSSA colonization in nares specimens was observed in older neonates (Mitsuda, Arai, Fujita, & Yokota, 1996). The prevalence of MSSA in umbilicus specimens in the present study was 1.7%; however, no published data are currently available for comparison. In this study, umbilical specimens were more likely to detect *S. aureus* than nasal swabs, and there was no transmission observed between MSSA positive pregnant women and their newborns. These results are contrary to those obtained by Mitsuda *et al.* who saw a greater sensitivity in nasal specimens compared to umbilicus swabs, and a 1.3% (4/305) MSSA transmission rate from mother to infant (Mitsuda, Arai, Fujita, & Yokota, 1996). It is possible that the collection of specimens at 6-days of age may be related to the colonization sites and rates observed by Mitsuda *et al.* (Mitsuda, Arai, Fujita, & Yokota, 1996).

Results obtained with the Kirby-Bauer disc diffusion method were equivalent to data obtained with the VITEK 2 Compact, with the exception that isolates that showed intermediate levofloxacin resistance with the VITEK 2 Compact were determined to be resistant with the Kirby-Bauer method. The reasons for the discrepancy are unknown.

MRSA infections that are acquired by persons who have not been recently hospitalized or have had a medical procedure are termed CA-MRSA infections. On the basis of questionnaire data, 4 of the 5 isolates in this study appear to be CA-MRSA. Strain typing using the bioMérieux DiversiLab® System database indicated that the 3 isolates with identical antimicrobial agent susceptibility profiles were most closely related to the Iberian MRSA strain. While the DiversiLab is useful for screening MRSA isolates, pulsed-field gel electrophoresis (PFGE) may be necessary for confirming specific USA types (Ross, Merz, Farkosh, & Carroll, 2005; Tenover et al., 2009). The participants in this study were representative of the pregnant women delivering vaginally at this university-affiliated clinic. From the questionnaire data, no statistically significant factors could be identified for MRSA colonization among

the pregnant women. This is in part due to the low colonization rates that provided very limited statistical power to evaluate risk factors. These low carriage rates have been observed in similar studies of vertical transmission (Reusch et al., 2008).

This is the first known survey of MRSA colonization of healthy neonates and pregnant women in Las Vegas, and the results will be useful in establishing a baseline to determine future increases in MRSA colonization rates among this population. It is expected that strain characterization of the MRSA positive isolates will help in developing recommendations for selecting antimicrobial therapy, and is the first step in establishing a pediatric database of the antimicrobial susceptibility of infection-causing microorganisms for all Las Vegas, Nevada hospitals.

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