

Nasal and vaginal colonization of methicillin-resistant *Staphylococcus aureus* in pregnant women in Cartagena-Colombia.

Colonización nasal y vaginal por *Staphylococcus aureus* resistente a metilina en mujeres embarazadas atendidas en la Clínica Rafael Calvo de Cartagena.

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Abstract

Introduction. The host niche for *Staphylococcus aureus* (SA) are the anterior nares; however, vaginal colonization rates between 14% - 17.1% in pregnant women have been reported recently, which raises the interest about the potential risk in postpartum women and in neonates from colonized mothers.

Objectives. To determine the prevalence of nasal and vaginal colonization of SA and the antibiotic susceptibility of the isolates in pregnant women attending a maternity hospital in Cartagena, Colombia.

Methods. Nasal and vaginal swabs were obtained from participants and subjected to microbiological and molecular assays. A post discharge follow-up was performed for up to four weeks. **Results.** From one hundred pregnant women enrolled in the study, 34 were colonized with SA; twenty nine only in the nares, three only in the vagina, and two at both sites. Colonization of pregnant women with SA was more common in the nares than in the vagina or at both sites [29/34 (85.3%) versus 3/34 (8.8%) and 2/34 (5.9%); $p < 0.05$]. We obtained 36 SA isolates, nine of which (25%) were MRSA, one was from the vagina; thus, the overall MRSA colonization rate among pregnant women was 9.0%. Molecular analysis showed that Panton-Valentine leukocidin (PVL) genes were carried by the vaginal MRSA, seven of the nasal MRSA, and two of the MSSA isolates. Two MRSA isolates carried SCCmec type I and seven carried SCCmec type IV. **Conclusions:** Nasal colonization rate for SA in the study population was similar to previous reports. However, the frequency of nasal colonization of MRSA was higher while vaginal colonization of SA was lower than previously reported in other studies for similar populations. MRSA isolates obtained showed a community profile.

Resumen

Introducción. *Staphylococcus aureus* (SA) ha adoptado como nicho habitual las narinas anteriores; sin embargo, recientemente se han reportado tasas de colonización del tracto genital de mujeres embarazadas entre 14%-17.1%, lo que acrecienta el interés respecto al riesgo asociado para el neonato y la madre en el posparto.

Objetivo. Determinar la prevalencia de colonización nasal y vaginal por SA y los patrones de susceptibilidad a antibióticos de los aislamientos en una comunidad obstétrica de Cartagena, Colombia.

Métodos. Hisopados nasales y vaginales fueron obtenidos de las participantes y sometidos a ensayos microbiológicos y moleculares. Se realizó seguimiento a la madre y al neonato durante cuatro semanas.

Resultados. De 100 embarazadas participantes 34 estuvieron colonizadas con SA; 29 solamente en fosas nasales, 3 solo vagina, y 2 en ambos sitios anatómicos. La colonización fue más común en fosas nasales que en vagina o en ambos sitios [29/34 (85.3%) versus 3/34 (8.8%) and 2/34 (5.9%); $p < 0.05$]. Se obtuvieron 36 aislamientos de SA de los cuales 9 fueron MRSA (25%), proviniendo uno de vagina; por lo tanto, la tasa de colonización total por MRSA en embarazadas fue 9.0%. Los genes para la leucocidina de Pantón-Valentine (PVL) se detectaron en la cepa MRSA vaginal, siete MRSA nasales, y dos MSSA. Dos aislamientos MRSA portaban el elemento SCCmec tipo I y siete el tipo IV. No se detectó resistencia a otros antibióticos en los aislamientos MRSA, pero tres aislamientos susceptibles a metilina (MSSA) fueron resistentes a eritromicina.

Conclusiones: Aunque la colonización nasal por SA en la población estudiada estuvo dentro del rango reportado previamente, la colonización nasal por MRSA fue mayor, mientras que la colonización vaginal fue más baja, que las reportadas previamente en otros estudios para poblaciones similares. Los aislamientos MRSA obtenidos presentaron un perfil comunitario.

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Introduction

Staphylococcus aureus (SA) is an important human pathogen responsible for nosocomial and community acquired infections, which also behaves as commensal flora in healthy individuals mainly colonizing the anterior nares¹. Carriers of SA, in particular Methicillin-resistant *Staphylococcus aureus* (MRSA), have a higher risk for developing clinical infections, being the infections caused by MRSA strains the most important at the clinical level, since they are more difficult to treat². In the last decades, MRSA strains, especially those associated to the community (Community associated Methicillin-resistant *Staphylococcus aureus*, CA-MRSA) have become an important issue in public health, since they are a cause of increased morbidity and mortality³. These CA-MRSA strains cause severe skin and soft tissue infections, necrotizing pneumonia and sepsis in otherwise healthy children, teens, and more recently, in neonates. The increasing number of infections in neonates caused by CA-MRSA, emphasizes the need to identify the possible sources of infection, either environmentally or maternally derived^{4,5}.

For pregnant women SA also pose a health risk, since it is the main cause of infection of the surgical site, causing between 25% to 50 % infections of the post cesarean surgical site, representing a major cause of morbidity and a cause of puerperal mastitis⁶. Early epidemiological studies showed that 5% of women were colonized with SA in their genital tract and postpartum women had the highest colonization rates; furthermore, vaginal-rectal carriage of SA has been found to be associated with development of postpartum fever⁷. Although risk factors associated to colonization with MRSA strains during pregnancy have not been fully characterized, associations with race, parity, type of birth, and colonization with group *B streptococci* have been suggested⁷. It is known that incidence of CA-MRSA infections vary among different communities and populations, and apparently, pregnant women are more susceptible and have risk factors that predispose them to develop these infections^{6, 8}. Despite of this, there is a scarcity of epidemiologic reports about MRSA infections presenting in pregnant and puerperal women⁶.

More recently, several studies, mainly from the United States, have reported vaginal colonization rates for SA in pregnant women from 14% to 17.1%, and although the risk of vertical transmission has been suggested or even demonstrated⁹⁻¹¹, the association of such coloni-

zation with infant outcome has not been clearly established¹².

Thus, there is a need to increase the knowledge in this area of research. A literature review of the subject reveals few published studies in this area, which are mainly reviews of clinical cases¹³, making clear that more epidemiology based studies are needed given the increasing importance of CA-MRSA strains in serious neonatal infections¹⁴ and in women in puerperal stage⁸.

Antibiotic resistance of MRSA strains is due to the acquisition of *mecA* gene, which codes for PBP-2a, a penicillin binding protein with low binding affinity for beta-lactam antibiotics. *MecA* gene is located in a mobile genetic element, the chromosomal staphylococcal cassette (SCCmec), of which seven major types have been identified, from I to VII. SCCmec types IV, V and VII are the most frequently found in CA-MRSA isolates, which also frequently carry the genes for Panton-Valentine leukocidin (PVL)¹⁵.

Up to 96% of MRSA infections in pregnant women mainly affect the skin and soft tissue, although several cases of pneumonia in the puerperal period have been also attributed to MRSA strains⁶. Since PVL-carrying MRSA strains have been implicated in the development of serious skin and soft tissue infections and in necrotizing pneumonia¹⁶, in the present study besides determining the SA nasal and vaginal colonization rates and antibiotic profiles of the isolates, we evaluated the presence of PVL genes and SCCmec types of MRSA isolates by multiplex PCR.

Methods

Design, Study Population, and Inclusion Criteria

The present was a pilot study performed by the Genetics and Molecular Biology Research Group of the University of Cartagena. The study site, Maternity Clinic Rafael Calvo (MCRC), in Cartagena, Colombia, is a university-affiliated maternity clinic attending pregnant women from the urban and rural areas around Cartagena, and whose obstetric service delivers between 8000 and 8500 infants per year. Following a research protocol approved by the Ethics Committee of University of Cartagena and the Institutional Review Board of MCRC, women with at least 35 weeks of gestational age, who attended the outpatient or urgency departments during the months of January through June 2009 were enrolled in the study

after signing an informed consent. Women with ruptured membranes and those under antibiotic treatment or with clinical evidence suggesting a current staphylococcal infection were excluded from the study. Following approaches previously described by Pinter et al¹³ and Beigi et al.⁹ nasal and vaginal swabs were obtained from the enrolled pregnant women (n = 100) with vaginal samples taken from the outer third portion of the vagina. Although the objective of the study was to determine SA nasal and vaginal colonization rates and antibiotic susceptibility, we tried to ascertain any possible associations of colonization with complications related to staphylococcal infection either in the mothers or their newborns implementing a post discharge weekly follow-up by telephone for up to four weeks. Mothers were contacted by telephone at least once a week during her baby's first month of life. They were asked about the general health of her infant as well as her own. Specific questions were asked to determine whether any fevers, rashes, or other skin lesions had occurred in the infant. Additionally, participants were asked to file a questionnaire about social demographic data and medical records.

Laboratory Methods

Nasal and vaginal specimens were transported to the microbiology laboratory of the School of Medicine at University of Cartagena, and processed within 8 to 18 hours according to protocols described by Bettin et al¹⁷. Antibiotic susceptibility of isolates confirmed as SA was performed by the disc diffusion method following recommendations of the Clinical and Laboratory Standards Institute (CLSI). Antibiotics evaluated were: rifampin (5 ug), clindamycin (2 ug), erythromycin (15 ug), gentamicin (10 ug), vancomycin (30 ug), cefoxitin (30 ug) and oxacillin (1 ug). SA strain ATCC 33591 was used as control. The D-zone test for inducible clindamycin resistance was performed for each isolate according to CLSI method. Isolates were classified as MRSA if they demonstrated resistance to cefoxitin and MSSA if they were susceptible. MRSA were further confirmed by PCR amplification of *mecA* gene. Both MSSA and MRSA isolates were stored at -35°C for subsequent molecular studies.

Molecular analysis

Genomic DNA extraction

Genomic DNA from each isolate was obtained according to the protocol described by Millar et al¹⁸ with some modifications. Briefly, each SA

isolate was subcultured in nutrient agar for 24h at 37°C. Around 5 colonies were resuspended in 1ml of Tris 0.5M, centrifuged at 13,000 rpm x 5min. Supernatant was discarded and the pellet resuspended in 500 ul buffer TE (10mM Tris; 1mM EDTA, pH:8.0) and boiled at 100°C for 30min, and then incubated at -35°C for 20 minutes, thawed at 65°C and finally centrifuged at 13.000 rpm for 15 minutes. Supernatant containing bacterial DNA was collected in a clean tube and stored at -20°C for subsequent PCR assays.

Multiplex PCR assays

Multiplex polymerase chain reaction assays for assessments of presence of the *lukF-PV* (encoding part of the PVL toxin), *mecA*, and *nuc* genes were performed for all SA isolates using previous described primers¹⁹⁻²¹. SA strains ATCC 33591 (*mecA* +; *nuc* +; PVL -) and ATCC 25923 (*mecA* -; *nuc* +; PVL +) were used as amplification controls and pure water as negative control. All SA isolates were subjected to multiplex PCR using a set of three primer pairs previously reported: *MecA1F* – *MecA2R* that amplifies a 147 bp fragment of *mecA* gene; *Nuc1F* – *Nuc2R* that amplifies a 300 bp fragment of *nuc* gene specific for SA, and *LukPV1F* – *LukPV2R* that amplifies a 437 bp fragment of PVL gene. Additionally, all confirmed MRSA isolates were subjected to SCCmec typing using a multiplex PCR assay according to the protocol described by Zhang et al.¹⁹ using SA strains NCTC10442 for SCCmec type I, N315 for SCCmec type II, and JCSC4744 for SCCmec type IV. PCR products were visualized in a 2% agarose gel stained with ethidium bromide under UV transillumination.

Statistical Analysis

Statistical analysis was performed with SPSS version 13.0 for Windows. Based on microbiological results, the study population was classified as carrier and non-carrier, and subclassified according to the colonization site (vagina/nares/both) and the frequency of colonization was determined for each of the two anatomical sites studied. Univariate analysis was applied to determine association of colonization to potential risk factors using the Fisher exact test using a p value ≤ 0.05 for statistical significance.

Results And Discussion

Table 1 shows demographic data from our study population and Table 2 shows the general results of SA isolates from this study. One hundred pregnant women were enrolled in this pilot study during a 6-month period and 34 of

Variables		Value
Age (years)	Mean	23,3
	SD	± 5
Gestational age (weeks)	Mean	37,7
	SD	± 2
Geographical origin	Urban	74
	Rural	26
Marital status	Single	13
	Married	10
	Unmarried partner	77
Scholarity	Primary	42
	Secondary	22
	Technical	21
	Universitary	4
	None	11
Parity	Nullipara	35
	Multipara	65

Table 1. General demographic characteristics of study population (n = 100), *SD* : standard deviation

them were colonized with SA. Twenty nine of them were colonized only in the nares, three were colonized only in the vagina, and two harbored *S. aureus* at both sites. Colonization of pregnant women with SA was more common in the nares than in the vagina or at both sites [29/34 (85.3%) versus 3/34 (8.8%) and 2/34 (5.9%); $p < 0.05$]. From the total of participants, we obtained 36 SA isolates, nine of which (25%) were MRSA, one was from the vagina; thus, the

overall MRSA colonization rate among pregnant women was 9.0%.

The nasal colonization rate for SA found in our pilot study (29%) was similar to rates previously reported for general population^{2,22}. However, the nasal colonization by MRSA (8% of the overall population of this study, 27.6% of the nasal SA isolates) was higher than the frequencies previously described both at international

Site	SA neg	SA pos (n=36)	
		MSSA	MRSA
Nasal	69	23	8
Vaginal	95	2	1
Both	98	2	0

Table 2. General results for *Staphylococcus aureus* isolates (n = 36) *MRSA : methicillin-resistant *Staphylococcus aureus* *MSSA : methicillin-sensitive *Staphylococcus aureus*. "Both" refers to cases in which *S. aureus* isolates were obtained from vagina and nares from the same patient.

and national level²². Albeit, at local level there is a report of similar nasal colonization rate for MRSA in scholar children²³ in other local report MRSA nasal isolates were no detected among elderly people residing in a nursing-home¹⁷. To our knowledge, the colonization for SA in pregnant women have not been evaluated previously in Colombia.

We found in this pilot study a 3% vaginal and 2% nasal and vaginal colonization rates for SA, which are comparable to rates reported in similar studies in geographically different populations^{9, 12}, and a 29% nasal colonization rate, which is in the range described in other similar studies^{9, 13}. For MRSA, nasal colonization rate in this group of pregnant women were much higher (8%) than the rates reported for the general population^{15,24} and for pregnant women in different geographical areas¹³. For example, a pilot study carried out in Cleveland-Ohio reported that 22% of the study population were nasal carriers of SA⁹. The authors of that study referenced a former report published in 1978 where the nasal colonization of SA in pregnant women was 4.0%. In a study similar to ours, Pinter et al. reported that from 304 pregnant women, 34 (11.2%) of them were colonized in the nares, 7 of whom (2.3%) were colonized with MRSA¹³.

Most recent data on SA colonization in obstetrics (both MSSA and MRSA) are derived mainly from the USA^{5,9-11,13} or Europe¹², and until now they provide little evidence that either universal or targeted screening is beneficial for mothers or babies^{5, 11, 13, 24, 25}. Higher colonization

rates with SA are observed when the samples are from rectal-vaginal samples^{10,11} compared to vaginal^{12,13}, which may reflect the fact that SA colonizes frequently the gastrointestinal tract. In the study published by Andrews et al.¹¹, genital tract colonization by MRSA among pregnant women was evaluated and correlated with infant outcome. From 5732 pregnant women participating in the study, 833 were colonized by SA (14.5%), 202 of whom were MRSA positive (3.5% overall MRSA colonization). The authors reported that no cases of early-onset invasive neonatal infection by MRSA occurred among infants in their study.

In the pilot study presented here, from 100 pregnant women screened, only 5 vaginal isolates were obtained, one of which was MRSA. Social demographic characteristics of our study population are summarized in Table 1. Statistical analysis of this data did not show significant associations with carrier state. This may reflect the fact that our sample population was small, although similar studies with larger populations have also failed to identify risk factors for SA colonization in pregnant women and the implications for maternal and neonatal outcomes^{5, 11, 12}. In our study, follow up could only be completed for 52 mother-infant pairs, and we did not observe SA related complications neither in mothers nor in newborns; thus, we could not identify factors associated to a higher risk of colonization or development of complications either in mothers or infants. To date, rectal vaginal colonization of SA has been associated to race, parity, route of birth, and colonization by *Streptococcus agalactiae*⁷. In fact,

<i>Characteristics</i>		<i>Number of isolates</i>
Origin	<i>Nasal</i>	8
	<i>Vaginal</i>	1
PVL* carrying	<i>Positive</i>	7
	<i>Negative</i>	2
SCCmec types	<i>Type I</i>	2
	<i>Type IV</i>	7

Table 3. Characteristics of Methicillin-Resistant *Staphylococcus aureus* isolates (n = 9) *PVL: Panton-Valentine Leukocidin *SCCmec: Staphylococcal Chromosomal Cassette *mec*.

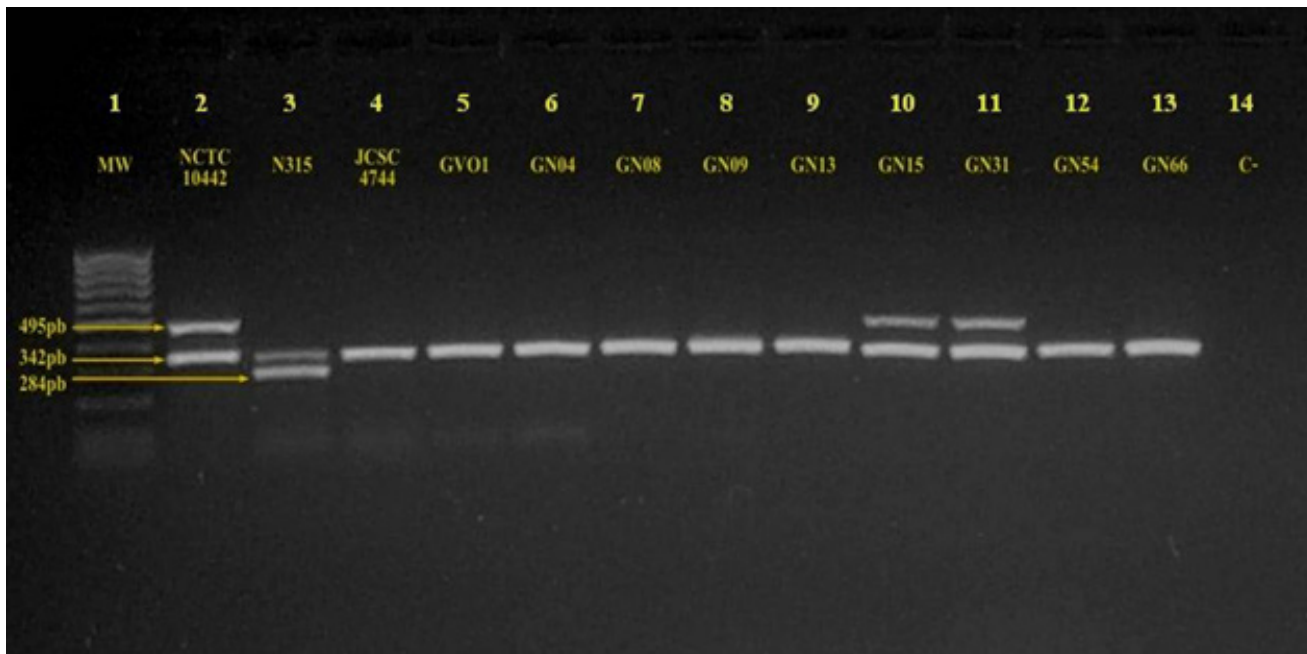


Figure 1. Multiplex PCR for SCCmec typing, Results for SCCmec typing by multiplex PCR. Lane 1: MW (DNA molecular weight marker). Lanes 2, 3, and 4 show results for SA strains NCTC10442 (SCCmec type I), N315 (SCCmec type II), and JCSC4744 (SCCmec IV). Lanes 5 to 13 show SCCmec typing results for MRSA isolates from the study. Two MRSA isolates were SCCmec type I (Lanes 10 and 11). Seven MRSA isolates were SCCmec type IV (Lanes 5 to 9 and Lanes 12 and 13). Lane 14 shows the results for a negative control of the PCR reactions.

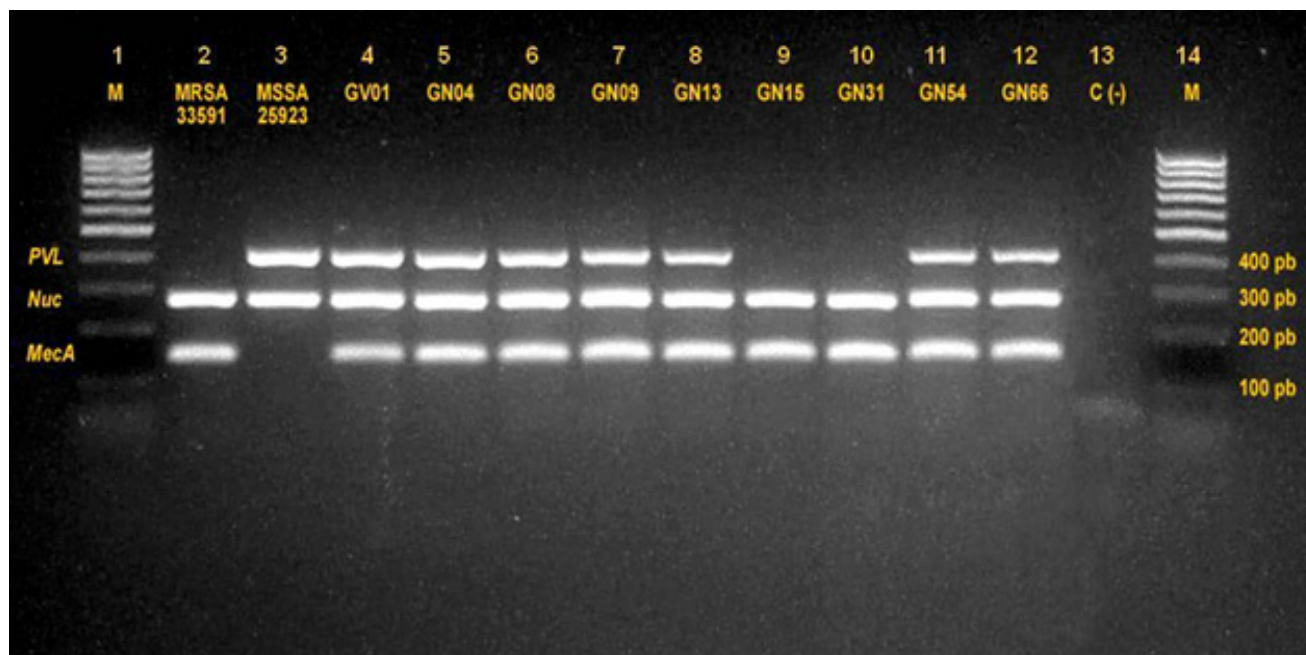


Figure 2. Multiplex PCR for detection of nuc, mecA, and PVL genes, Multiplex PCR was carried out to detect the presence of nuc, mecA and PVL genes in isolates from pregnant women to confirm *Staphylococcus aureus* species (nuc gene), methicillin resistance (mecA gene) and presence of PVL genes. Lanes 1 and 14: M (DNA molecular weight markers). Lanes 2 and 3 : ATCC 33591 reference strain (nuc+, mecA+, PVL-) and ATCC 25923 reference strain (nuc+, mecA-, PVL+) respectively. Lanes 4 to 12: representative isolates from the study. Lane 13: negative control of the PCR reactions.

Chen et al. reported that patients that were colonized by *S. agalactiae* were also more likely to be colonized with SA, but those colonized by MRSA were not colonized with *S. agalactiae*⁷.

It is of interest to compare our current results for maternal colonization with SA and MRSA with those of Pinter et al.¹³, who reported a maternal colonization rate of 14.1% for SA and of 3% for MRSA, much lower to the reported in the current study. It should be noted, for comparison, that in the study by Pinter et al. all participants delivering their babies by the vaginal route were screened on the day of delivery at both the nares and the vagina in contrast with the current study in which pregnant women were screened at both the nares and vagina between 35 and 42 weeks of gestational age, 17% of whom were in labor at the time of sampling. Whether differences in the colonization rates are related to differences in geographical areas or in the methodology used is uncertain.

Although asymptomatic nasal, vaginal, and rectal colonization with MRSA have been reported to occur in some pregnancy-related clinical cases in which SA colonization has been identified as risk factors for serious systemic infection after delivery⁸, epidemiological studies show that universal screening and decolonization efforts in pregnant women are currently non cost-effective^{2, 25}. Similarly, there is no current evidence that vertical transmission of SA constitute a significant risk for neonatal complications, although vertical transmission of SA have been documented in some studies^{4, 13}.

In our pilot study, antibiotic susceptibility of the SA isolates found that all MRSA isolates had the resistance profile associated to the community, with exclusive resistance to methicillin. Also, three MSSA isolates had erythromycin resistance; one of them had the erythromycin inducible clindamycin resistance phenotype, detected by the D-test. We did not find resistance to other antibiotics neither in MRSA nor MSSA isolates.

Table 3 summarizes the characteristics of MRSA isolates. SCCmec typing by multiplex PCR assay (Figure 1), showed that among the MRSA isolates from this study, two had SCCmec type I and seven had SCCmec type IV, data consistent with community-associated MRSA since it has been previously reported that MRSA isolates commonly found in community settings frequently bear SCCmec type IV (and V)¹⁵.

Although controversial, Panton-Valentine leukocidin has been widely regarded as a major virulence determinant associated to epidemic clones of CA-MRSA in the world¹⁶. In the current study we determined the presence of PVL genes among SA isolates, both MSSA and MRSA. Among MRSA isolates, six nasal isolates and the vaginal isolate were PVL positive (Figure 2). In contrast, among the MSSA isolates, only two were PVL positive; thus, PVL genes were more frequently found in MRSA isolates compared to MSSA [7/9(77.7%) versus 2/27(7.4%); $p < 0.01$].

Conclusions

Although the current was a small pilot study and it may not be generalized to the entire pregnant population, the colonization rates found for MRSA were high, as were high the frequency of PVL positive MRSA isolates, emphasizing the need for further epidemiologic studies addressed to evaluate the impact of this colonization in puerperal and neonatal morbidity in our geographical region and to identify subpopulations of pregnant women that may benefit from SA screening, since morbidity and associated costs for MRSA infections in this population are increasingly reported^{6, 8}.

We could not find statistical significance among socio demographic factors, colonization status and development of complications, which could be attributed to the low number of participants able to complete the follow up. Thus, additional studies with more epidemiologic power are needed to ascertain the role of SA colonization in pregnant women from our region.

Antibiotic susceptibility profiles, SCCmec typing, and presence of PVL genes of MRSA isolates points out to their community origin.

Conflict Of Interest

The authors declare that they do not have any conflict of interest related to this study.

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