Vagina is not a significant reservoir of methicillin-resistant 
*S. aureus* in pregnancy

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Abstract

Presence of *Staphylococcus aureus* in the pregnant woman's vagina may contribute to the development of various life-threatening infections. The risk of perinatal transmission of *S. aureus* into the newborn cannot be excluded. MRSA strains are acknowledged to be the alert pathogens responsible for both hospital-acquired and community-acquired infections necessitating restrictive epidemiological monitoring. The aim of the present study was to determine the drug susceptibility of *S. aureus* strains isolated between June 2009 and June 2013 from the vaginal swabs coming from pregnant women. The disk-diffusion method was used to investigate bacterial susceptibility to 14 antistaphylococcal agents. MICs of cefoxitin, vancomycin, and daptomycin were carried out with the use of the E-test method. The presence of the *mecA* gene was verified by multiplex PCR. The identification of the SCCmec types in MRSA strains was performed. The vaginal *S. aureus* strains isolated in pregnancy turned out to be highly susceptible to the antistaphylococcal agents available. Resistance to methicillin was a rare feature of the bacterial isolates tested. Vaginal MRSA isolates coming from pregnant women presented features typical of community-acquired rather than nosocomial staphylococci.

Key words: *Staphylococcus aureus*, MRSA, vagina, pregnancy

Introduction

*S. aureus* is an important human pathogen that causes diseases ranging from relatively mild infections of the skin and soft tissue to life-threatening systemic infections. According to the report presented by the Centers for Disease Control and Prevention (CDC) in 2007, it is the leading cause of serious and fatal infectious diseases in the USA [1].

There are three main reasons underlying this state of affairs. First of all, *S. aureus* can become a versatile pathogen causing a broad spectrum of infections thanks to a large arsenal of virulence factors [2, 3]. Secondly, these bacteria have become the urgent present-day epidemiological problem, not only in the hospital settings but also among the outpatients, because of their extraordinary ability to develop numerous mechanisms of antimicrobial drug resistance. It is particularly hard to fight against infections caused by *S. aureus* strains resistant to both beta-lactam antibiotics stable in the presence of staphylococcal penicillinases and glycopeptides [3, 4]. Finally, *S. aureus* is a common commensal organism on the human skin and mucosal surfaces. It has been estimated that as many as 40-50% of adults are either persistent or intermittent *S. aureus* carriers in the anterior nares. Vagina is one of the anatomic locations colonized by *S. aureus* [5, 6].

Vaginal biocenosis is a complex population of microorganisms covering the vaginal mucosa. Its qualitative and quantitative composition as well as the interactions between all microorganisms present in the vagina play a decisive role for the proper functioning of the organ, and the overall woman health [7, 8]. Presence of *S. aureus* in the pregnant woman’s vagina may contribute to the development of various life-threatening infections [9, 10]. Moreover, the risk of perinatal transmission of *S. aureus* into the newborn cannot be excluded. Colonization with MRSA (methicillin-resistant *S. aureus*) and VRSA (vancomycin-resistant *S. aureus*) strains is especially worrying [5, 11]. These *S. aureus* strains are acknowledged to be the alert pathogens responsible for both hospital-acquired and community-acquired infections necessitating restrictive epidemiological monitoring [12].

The aim of the present study was to determine the drug susceptibility of *S. aureus* strains (with particular emphasis on the staphylococcal alert pathogens) isolated between June 2009 and June 2013 from the vaginal swabs coming from pregnant women.

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Material

The study included 106 *S. aureus* strains comming from pregnant women hospitalized at the Polish Mother’s Health Center and the Madurowicz Regional Specialist Hospital in Lodz, the third largest city in Poland, Eastern Europe, between June 2009 and June 2013.

Methods

Vaginal swabs were collected using swabs with Amies transport medium (Argenta, Poznan, Poland). After 48-hour incubation at 37°C bacterial isolates were identified on the basis of their macroscopic and microscopic morphology (growth on the blood agar and mannitol salt agar, Gram staining), and slide and tube coagulase tests (bioMérieux, Warsaw, Poland). Biochemical properties of each confirmed isolate were determined using API 20 Staph tests (bioMérieux, Warsaw, Poland).

Antimicrobial susceptibility testing was performed in accordance with the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [13]. The disk diffusion method was used to investigate bacterial susceptibility to the following antimicrobial agents: cefoxitin (FOX, 30 μg), penicillin (P, 10 IU), erythromycin (E, 15 μg), clindamycin (DA, 2 μg), tetracycline (TE, 30 μg), ciprofloxacin (CIP, 5 μg), gentamicin (CN, 10 μg), trimethoprim-sulphamethoxazole (cotrimoxazole; SXT, 1.25/23.75 μg), linezolid (LZD, 30 μg), fusidic acid (FD, 10 μg), and quinupristin-dalfopristin (QD, 15 μg) (Oxoid, Poznan, Poland); tigecycline (TGC, 15 μg), chloramphenicol (C, 30 μg), rifampin (RA, 5 μg) (Bio-Rad, Warsaw, Poland). Susceptibility to vancomycin (VA256) and daptomycin (DPC256) (BioMérieux, Warsaw, Poland) was carried out using E-test method. This technique was also used to determine minimal inhibitory concentration (MIC) value for cefoxitin (FX256) (BioMérieux, Warsaw, Poland). Bacterial isolates were inoculated on Mueller-Hinton II Agar (bioMérieux, Warsaw, Poland) and incubated in aerobic atmosphere at 35°C for 18 hours (24 hours for cefoxitin and glycopeptides). The drug susceptibility results were interpreted as previously described [14].

Molecular confirmation of resistance to cefoxitin was performed then. The total bacterial DNA was extracted from all isolates by enzymatic lysis using the buffers and solutions provided with the Gene MATRIX Tissue & Bacterial DNA Purification Kit (EURx, Gdansk, Poland) according to the manufacturer’s instruction with 4U lysostaphin (A&A Biotechnology, Gdynia, Poland). The presence of the mecA gene was verified by the multiplex polymerase chain reaction (multiplex PCR). Multiplex PCR assays were performed in 20 μl reaction mixture containing 0.5 U KAPA® Taq DNA Polymerase (KAPABiosystems, Boston, USA), 1x polymerase buffer, 250 μM dNTPmix (Bioron GmbH, German), and 0.25 μM of primers (Sigma). The presence of the mecA gene was checked with primers: 5’tagaatgactgaagctcgatt3’ and 5’ccatctccatggttcg3’ (product size – 310 bp) [15]. DNA amplification was carried out in a GeneAmp PCR system 9700 (Applied Biosystems, USA) with the following thermal cycling profile: an initial denaturation step at 94°C for 4 min was followed by 30 cycles of 30 s at 94°C, 30 s at 55°C and 60 s at 72°C, with a final extension to 7 min at 72°C. PCR products were analyzed by Microchip Electrophoresis System MultiNA (Shimadzu). The SCCmec (staphylococcal cassette chromosome mec) types in all mecA-positive *S. aureus* isolates were identified as previously described [16].

The study was approved by the Bioethics Committee at the Medical University of Lodz (approval RNN/475/10/KB).

Results

Overall, drug susceptibility of 106 *S. aureus* strains was analysed. The detailed susceptibility of all clinical strains to the antimicrobial agents tested is shown in Table 1.

Resistance to cefoxitin (standing for methicillin resistance, MRSA) was found only in 4 bacterial isolates (3.77%) in comparison to 102 cefoxitin-susceptible strains (96.23%). These results were fully confirmed by both E-test method and PCR technique. The MIC value for cefoxitin ranged from 8 μg/ml to 12 μg/ml and from 1.5 μg/ml to 3 μg/ml for MRSA and MSSA isolates, respectively. The mecA gene determining staphylococcal resistance to all beta-lactam antibiotics was found only in the cefoxitin-resistant strains (4 isolates). Most *S. aureus* strains studied were resistant to penicillin (59 strains, 55.66%). Resistance to ciprofloxacin, tetracycline, and chloramphenicol was found in 12 strains (11.32%), 8 strains (7.54%), and 2 strains (1.88%), respectively. Resistance to erythromycin and clindamycin was revealed in 14 (13.20%) and 10 (9.43%) *S. aureus* isolates, respectively. The tested *S. aureus* strains were entirely susceptible to gentamicin, tigecycline, rifampin, trimethoprim-sulphamethoxazole (cotrimoxazole), linezolid, fusidic acid, quinupristin-dalfopristin, vancomycin (MIC < 2 μg/ml), and daptomycin (MIC < 1 μg/ml). Twenty four bacterial isolates (22.64%) proved to be susceptible to all antistaphylococcal agents studied.
Table 1. Drug susceptibility of *S. aureus* strains isolated from the vagina of pregnant women

<table>
<thead>
<tr>
<th>Antistaphylococcal agent</th>
<th>S. aureus strains (N = 106)</th>
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<tbody>
<tr>
<td></td>
<td>Resistant</td>
<td>Susceptible</td>
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<tr>
<td></td>
<td>n</td>
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<tr>
<td>Cefoxitin</td>
<td>4</td>
<td>3.77</td>
<td>102</td>
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<tr>
<td>Penicillin</td>
<td>59</td>
<td>55.66</td>
<td>47</td>
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<tr>
<td>Erythromycin</td>
<td>14</td>
<td>13.20</td>
<td>92</td>
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<tr>
<td>Clindamycin</td>
<td>10</td>
<td>9.43</td>
<td>96</td>
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<tr>
<td>Ciprofloxacin</td>
<td>12</td>
<td>11.32</td>
<td>94</td>
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<tr>
<td>Tetracycline</td>
<td>8</td>
<td>7.54</td>
<td>98</td>
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<tr>
<td>Chloramphenicol</td>
<td>2</td>
<td>1.88</td>
<td>104</td>
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<tr>
<td>Cotrimoxazole</td>
<td>0</td>
<td>0.00</td>
<td>106</td>
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<tr>
<td>Gentamicin</td>
<td>0</td>
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<td>106</td>
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<tr>
<td>Rifampin</td>
<td>0</td>
<td>0.00</td>
<td>106</td>
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<tr>
<td>Fusidic acid</td>
<td>0</td>
<td>0.00</td>
<td>106</td>
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<tr>
<td>Quinupristin-dalfopristin</td>
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<td>Tigecycline</td>
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<td>Linezolid</td>
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<td>Daptomycin</td>
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<tr>
<td>Vancomycin</td>
<td>0</td>
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The SCCmec types in four mecA-positive *S. aureus* strains were identified (Fig. 1). Two of them carried the SCCmec type IV and presented phenotypic resistance to beta-lactam antibiotics, macrolides, lincosamides, and tetracyclines. And the next two MRSA isolates proved to be positive for the SCCmec type V, showing phenotypic resistance only to beta-lactam antibiotics and tetracyclines.

![Fig. 1. PCR amplification products obtained with SCCmec types IV (line 3 and 4) and V (line 1 and 2)](bp)

**Discussion**

The normal microflora of the vagina mainly consists of *Lactobacillus* sp. and *Bifidobacterium* sp. Many other bacterial genera are involved in this complex ecosystem, including staphylococci, streptococci, enterococci, coryneforms, and *Enterobacteriaceae* members [7, 17].

*S. aureus* is a frequent causative agent of skin and soft tissue infections as well as life-threatening bloodstream infections. Less well known is *S. aureus* vaginal colonization. This bacterium has been reported to colonize the vagina in 4-22% of pregnant women [18, 21]. Andrews et al. [22] worked on the most sensitive strategy for the detection of *S. aureus* among pregnant women and newborn infants. The authors have determined the sensitivities of several body sites being the potential *S. aureus* reservoirs. Vagina was characterized by the lowest sensitivity for *S. aureus* detection (8%) comparing to nares, throat, rectum, and skin (52%, 50%, 13%, and 10%, respectively). Sampling nares and throat was considered to be essential to the identification of *S. aureus* carriers among both pregnant women and neonates [22].

As more and more *S. aureus* strains become the alert pathogens due to their increasing and complex resistance to antistaphylococcal agents available today, we decided to determine the drug susceptibility of *S. aureus* strains isolated from the vagina of pregnant women, the potential reservoir of staphylococcal pathogens for the newborns. One hundred and six *S. aureus* isolates have been collected for 4 years. Surprisingly, only four vaginal isolates (3.77%) turned out to be methicillin-resistant (so-called MRSA isolates). Most *S. aureus* strains (96.23%) were susceptible to beta-lactam antibiotics (so-called MSSA isolates). Moreover, all clinical *S. aureus* strains, including MRSA isolates, presented high susceptibility to the antistaphylococcal drugs tested.

Andrews et al. [18], who estimated the frequency of genital tract colonization by MRSA strains among pregnant women and evaluated the association of such colonization with infant outcome, have concluded that the genital tract colonization with MRSA affected 3.5% of pregnant women. In the study presented by Creech et al. [21], clinical samples from 250 pregnant women undergoing screening for recto-vaginal group B streptococcus colonization were evaluated for the presence of MRSA. *S. aureus* was detected in 21.6% of the women and 53.7% of the isolates were MRSA. Chen et al. [20] have been estimating the extent of methicillin-sensitive and methicillin-resistant *S. aureus* vaginal-rectal colonization among pregnant women. Only 17.1% of 2963 recto-vaginal swabs studied were positive for *S. aureus*. Of the *S. aureus* isolates, 14 (2.8%) were methicillin-re-
sistant, and 13 of these were determined to be community-associated MRSA. Extremely low recto-vaginal *S. aureus* colonization percentage (1.0%) among pregnant women was also described by Ghanim et al. [23]. *S. aureus* did not predispose to maternal or infant morbidity or mortality up to 6 months postpartum, as the authors claimed. Tomlinson et al. [24] have determined the rate of *S. aureus* recto-vaginal colonization and positive for *S. aureus* newborn blood cultures. Only 8.2% of 1488 recto-vaginal cultures were positive for *S. aureus*, among which 1.7% amounted MRSA isolates. Many American studies have reported prevalence ranging from 0.47% to 3.5% with community acquired MRSA in the obstetric population [25]. Bourgeois-Nicolaos et al. [26] have estimated that the prevalence of vaginal carriage of *S. aureus* was 5.9% among 1139 pregnant women within 1 month of delivery. The colonization rate of *S. aureus* in newborns was tenfold higher when the mother was a vaginal carrier than when she was not. It is worth noting that no *S. aureus* colonization was detected in the mothers of 58% of the colonized newborns suggesting extra-delivery colonization routes [26]. The study carried out by Chatzakis et al. [27] confirm that the maternal carriage of *S. aureus* play a principal role in infant colonization.

Vertical transmission from mothers to infants at delivery has been proposed as a possible mechanism of acquisition of community-acquired MRSA [28]. Although, the risk of horizontal rather than vertical *S. aureus* transmission from mothers to newborns has been described [24, 29]. All MRSA strains characterized in our study seem to be community-acquired due to their SCCmec type (IV or V) and lack of multidrug resistance. Low level of resistance to the drugs with antistaphylococcal potency is typical of the community-acquired infections rather than nosocomial infection, with the exception of the limited outbreaks of multi-drug resistant community-acquired MRSA [30]. Hospital-acquired MRSA strains typically have SCCmec types I, II and III while community-acquired MRSA isolates carry types IV, V or VII of the SCCmec fragment [31, 32]. The only antistaphylococcal drugs against which the vaginal *S. aureus* strains characterized in the present study revealed relatively high resistance were classic beta-lactam antibiotics (55.66%). However, such a tendency disqualifying penicillins from the antistaphylococcal therapeutic options has been observed since the 1950s [33].

The consequences of the maternal MRSA carriage for newborns stay unclear and difficult to be estimated due to relatively uncommon *S. aureus* recto-vaginal colonization in pregnancy. However, it does not appear to pose a significant risk of early onset neonatal sepsis [18, 24, 26]. Chen et al. [20] recommend the continued monitoring of both MSSA and MRSA infections among pregnant women and their infants. Recognizing the vagina as the *S. aureus* reservoir is important in women presenting with recurrent genital infections [9].

To summarize, several anatomic locations have been described as the stamping ground for *S. aureus* colonization. Vagina is one of them. Our work, aimed at characterizing the drug susceptibility of coagulase-positive staphylococcal strains isolated from vaginal swabs of pregnant women, confirm that in this approach *S. aureus* exemplifies a slight microbial threat for both the mother and the child due to its high susceptibility to antistaphylococcal drugs and epidemiological characteristics. Although the isolation of MRSA strain from the vagina in pregnancy should not be ignored.

References


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