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Jonathan C. Gullett, Emory University
Lars Westblade, Emory University
Daniel A. Green, Columbia University Medical Center
Susan Whittier, Columbia University Medical Center
Eileen Burd, Emory University

Journal Title: Journal of Clinical Microbiology
Volume: Volume 55, Number 6
Publisher: American Society for Microbiology | 2017-06-01, Pages 1604-1607
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1128/JCM.02387-16
Permanent URL: https://pid.emory.edu/ark:/25593/s6rx6

Final published version: http://dx.doi.org/10.1128/JCM.02387-16

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Accessed June 17, 2018 11:33 PM EDT
The Brief Case: Too Beta To Be a “B”

Jonathan C. Gullett,a Lars F. Westblade,b Daniel A. Green,c Susan Whittier,c Eileen M. Burda,d,e

Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, Georgia, USAa; Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York, New York, USAb; Department of Pathology and Cell Biology, Columbia University Medical Center, New York, New York, USAc; Department of Medicine, Division of Infectious Diseases, Emory University School of Medicine, Atlanta, Georgia, USA; Emory Antibiotic Resistance Center, Emory University School of Medicine, Atlanta, Georgia, USAe

KEYWORDS Streptococcus pseudoporcinus, adverse obstetrical outcomes, betahemolytic streptococci, group B streptococci

CASE

A 29-year-old African-American woman was seen in her third pregnancy. In her first pregnancy, she experienced preterm labor and premature rupture of membranes with delivery of a preivable infant at 5 months. Her second pregnancy ended in spontaneous abortion at 7 weeks. Current other medical conditions include obesity, chronic kidney disease, benign essential hypertension, and prediabetes. She has a history of abnormal cervical cytology smears and was treated with colposcopy and a loop electrosurgical excision procedure, which was followed by successive normal cytology exams. Because of her history of pregnancy complications and short cervical length (<2 cm), her current pregnancy was managed with placement of a prophylactic McDonald cerclage at the gestational age of 21 weeks. The cerclage was removed at gestational age 37 weeks. The mother was monitored for slow fetal growth in the third trimester that was suspected to be due to placental insufficiency. A vaginorectal culture taken at 39 weeks for Streptococcus agalactiae (group B Streptococcus) grew a beta-hemolytic organism with a wide zone of hemolysis (Fig. 1). Gram staining showed Gram-positive cocci in pairs and short chains (Fig. 1), and the organism was catalase negative and pyrrolidonyl arylamidase (PYR) negative and produced a weak positive reaction with the group B latex typing reagent in the PathoDx Strep grouping kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Because the wide zone of beta-hemolysis was not consistent with S. agalactiae, the isolate was analyzed using Vitek MS matrix-assisted laser desorption–ionization time of flight mass spectrometry (MALDI-TOF MS) (bioMérieux, Durham, NC, USA), which returned a nonclinically validated identification of Streptococcus porcinus, with a confidence score of 99.9% generated using IVD Knowledge Base version 2.0 and Myla version 4 information management software. To corroborate the accuracy of the MALDI-TOF MS result, the 16S rRNA gene was amplified using PCR and the resultant DNA fragment sequenced. Sequence data were analyzed using BLAST (https://blast.ncbi.nlm.nih.gov [16S rRNA sequence database]) and yielded 99% sequence homology (1,443 nucleotides [nt]/1,458 nt) to the Streptococcus pseudoporcinus LQ 940-04T strain. The next-best matches, all with 96.7% homology, were to S. porcinus 176T (1,450 nt/1,499 nt), Streptococcus hongkongensis HKU30T (1,370 nt/1,417 nt), and Streptococcus uberis JCM 5709T (1,455 nt/1,505 nt). These data supported an identification of S. pseudoporcinus rather than S. porcinus.

The patient required Cesarean delivery at the gestational age of 39.3 weeks after failed induction of labor for severe gestational hypertension/preeclampsia. In this case, there was no premature rupture of membranes, and no intrapartum antibiotics were administered. On gross examination, the placenta weighed 383 g (<10th percentile for


Editor Carey-Ann D. Burnham, Washington University School of Medicine

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Address correspondence to Eileen M. Burd, eburd@emory.edu.

For answers to the self-assessment questions and take-home points, see page 1973 in this issue (https://doi.org/10.1128/JCM.02390-16).
gestational age) and had increased perivillous fibrin. Both the patient and the infant did well subsequently.

**DISCUSSION**

*Streptococcus porcinus* is a beta-hemolytic *Streptococcus* species with Lancefield group NG1 (A1, C1), NG2, NG3, E, P, U, or V antigen, although some strains may be untypeable (1). It was first isolated from swine in 1937 and was later found to be associated with sheep, rabbits, dogs, guinea pigs, and cattle. In pigs, *S. porcinus* has been shown to colonize the genital and upper respiratory tracts and is implicated in cellulitis, endocarditis, sepsis, cervical lymphadenitis, and spontaneous abortion (1, 2).

Human sources of what appeared to be *S. porcinus* were first described in 1995. In 2005, Duarte et al. used randomly amplified polymorphic DNA-PCR and pulsed-field gel electrophoresis analyses to show that these human isolates differed from isolates obtained from animal sources, with the majority of the human strains containing NG1 antigen (3). Subsequently, analysis of 16S rRNA gene sequence data of several human isolates recovered from female genitourinary tract specimens phenotypically described as *S. porcinus* revealed the sequences to be >2% dissimilar to any other *Streptococcus* species and thus highly suggestive of a novel species (4). These findings led to the creation of a new species termed *Streptococcus pseudoporcinus*.

*S. pseudoporcinus* is a facultative, nonmotile, Gram-positive coccus that produces smooth, round-to-oval, beta-hemolytic colonies on blood agar (Fig. 1) and may possess E, P, or NG1 antigen or be untypeable (1, 3, 5). Like *S. agalactiae*, *S. pseudoporcinus* produces large colonies (typically >0.5 mm) after 24 h of incubation (which is useful for differentiating beta-hemolytic streptococci prior to Lancefield antigen typing because it excludes the possibility of beta-hemolytic strains of members of the *Streptococcus anginosus* group, whose colonies are characteristically smaller [≤0.5 mm]), is CAMP factor positive, and is bacitracin resistant, and both species are most commonly isolated from the female genitourinary tract (1, 2). Although, unlike *S. agalactiae*, *S. pseudoporcinus* does not contain Lancefield group B antigen, it cross-reacts with group B antigen agglutination reagents in *Streptococcus* serogrouping kits (1, 4–8). Furthermore, colonies of *S. pseudoporcinus* have been reported as indistinguishable from those of *S. agalactiae* when chromogenic agars designed for the detection of *S. agalactiae* are utilized (6). Taken together, this can make differentiation between *S. pseudoporcinus*

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**FIG 1** (A) A Gram stain of a *Streptococcus pseudoporcinus* colony shows Gram-positive cocci in pairs, short chains, and entwined chains (original magnification, ×1,000; oil immersion). (B and C) *Streptococcus pseudoporcinus* colony morphology after overnight incubation on tryptic soy agar with 5% sheep blood (Thermo Fisher Scientific [Remel], Lenexa, KS, USA) at 35°C in 5% carbon dioxide, demonstrating a large zone of beta-hemolysis (B) in contrast to the narrow zone of beta-hemolysis characteristic of *Streptococcus agalactiae* (group B *Streptococcus*) after overnight incubation on tryptic soy agar with 5% sheep blood at 35°C in 5% carbon dioxide (C).
and *S. agalactiae* difficult and has probably led to an underestimation of the former’s role and frequency in human colonization and disease. Frequency may also be underestimated because molecular screening tests to detect *S. agalactiae* do not detect *S. pseudoporcinus* (6).

In contrast to *S. agalactiae*, which displays a narrow zone of beta-hemolysis, *S. pseudoporcinus* exhibits a large zone (Fig. 1). Additionally, *S. agalactiae* is esculin hydrolysis negative and hippurate hydrolysis positive and does not ferment sorbitol or mannitol, while the opposite is true for *S. pseudoporcinus* (1, 3, 4). Also, *S. agalactiae* is PYR negative, while some varied PYR results have been reported for *S. pseudoporcinus* and *S. porcinus* (4). Finally, while MALDI-TOF MS is a robust and reliable identification tool, it produced an identification of *S. porcinus* in this case since the closely related *S. pseudoporcinus* is not included in the Vitek MS knowledge database but is planned for a future version. As a point of reference, our isolate when analyzed using the Bruker Biotyper platform (Bruker Daltonics, Billerica, MA) and prepared using the extended direct transfer method (application of the organism to the target and overlay with 70% [vol/vol] formic acid prior to addition of matrix) was identified as *S. porcinus*, with a log(score) value of 1.914 (probable genus identification) using the research-use-only database (RUO6903). When prepared using the ethanol-formic acid extraction method to increase the efficiency of protein extraction, the isolate was identified as *S. porcinus* with a log(score) value of 2.142 (secure genus identification, probable species identification), again using the RUO6903 database.

Though little is known about the pathogenicity of *S. pseudoporcinus* in human disease, the literature suggests the possibility of adverse obstetric outcomes. For example, while it has not been implicated as a cause of neonatal sepsis, it has been associated with chorioamnionitis and preterm delivery (1). The underlying pathophysiology appears to be cervical as opposed to uterine. Some studies suggest that an ascending *S. pseudoporcinus* infection may trigger an inflammatory cascade, leading to cervical and amniotic membrane microarchitecture remodeling and ultimately leading to cervical insufficiency or premature rupture of membranes (8). Although the clinical significance of *S. pseudoporcinus* has not been completely established, we report the identification when we recover the organism from *S. agalactiae* screening cultures. It may actually be more useful when it is detected early in pregnancy, since some of the conditions potentially attributed to this bacterium, such as chorioamnionitis, can be experienced as early as the first trimester.

The acquisition of *S. pseudoporcinus* has been independently associated with black women, aged 21 to 43 years, who have ever reported genital herpes simplex virus infection, recent *Trichomonas vaginalis* infection, bacterial vaginosis by the Nugent criteria, or obesity or who have had two or more male sexual partners during the 1 or 2 months since their last health clinic visit (i.e., increased numbers of sexual partners) (1, 6). Therefore, based on some of these findings, it has been suggested that *S. pseudoporcinus* may be associated with sexually transmitted infections (STIs). *S. pseudoporcinus* is exquisitely susceptible to beta-lactam antibiotics, vancomycin, and trimethoprim-sulfamethoxazole but has a high rate of resistance to tetracycline (1, 3, 7). Although some isolates of *S. pseudoporcinus* are resistant to erythromycin and clindamycin, significantly higher rates of resistance have been observed with *S. agalactiae* (1, 3, 5, 7).

In conclusion, the increase in the number of isolates identified as *S. pseudoporcinus* is most likely related to improvements in identification methods and greater awareness of this organism rather than its recent emergence in the human population (5). The association between *S. pseudoporcinus* and ethnicity, STIs, and adverse obstetrical outcomes warrants further investigation. Controlled studies in which microbiologic testing is performed to distinguish between *S. agalactiae* and *S. pseudoporcinus* are needed in order to determine if the latter is consistently identified as the true causative agent in morbid pregnancy-related outcomes.
SELF-ASSESSMENT QUESTIONS

1. Which of the following characteristics suggests that an isolate of Gram-positive cocci in short chains that is catalase negative and Lancefield group B antigen positive may be *Streptococcus pseudoporcinus*?
   - A. A narrow zone of beta-hemolysis, esculin hydrolysis negativity, and hippurate positivity
   - B. A narrow zone of beta-hemolysis, esculin hydrolysis positivity, and hippurate positivity
   - C. A wide zone of beta-hemolysis, esculin hydrolysis positivity, and hippurate negativity
   - D. A wide zone of beta-hemolysis, esculin hydrolysis negativity, and hippurate positivity

2. What is the normal habitat for *Streptococcus pseudoporcinus* in humans?
   - A. Vagina
   - B. Skin
   - C. Mouth
   - D. Conjunctiva

3. *Streptococcus pseudoporcinus* is possibly associated with which of the following adverse obstetrical outcomes?
   - A. Neural tube defects
   - B. Neonatal sepsis
   - C. Pregnancy-induced hypertension
   - D. Premature rupture of fetal membranes and cervical insufficiency

REFERENCES


