Molecular and antimicrobial susceptibility characterization of \textit{Globicatella sulfidifaciens} isolated from sow’s urinary tract infection

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\textbf{ABSTRACT}

\textbf{Background:} The \textit{Globicatella} genus comprises Gram-positive, facultative anaerobic, \(\alpha\)-hemolytic and catalase negative cocci morphologically and phenotypically very similar to \textit{Streptococcus} and \textit{Aerococcus} genus which can lead to misidentification and underestimation of this pathogen. \textit{Globicatella} species have already been isolated from human and animals with heart and brain disorders. Their clinical relevance in animals, and its zoonotic potential, remains unknown due to the difficulty in their identification.

\textbf{Objective:} To present the isolation, phenotypic and molecular characterization of \textit{G. sulfidifaciens} from urinary tract infection in sows.

\textbf{Materials and Methods:} Urine samples from 140 sows of two swine herds located in São Paulo State (Brazil) yielded the isolation of three presumptive \textit{G. sulfidifaciens} strains. Identification and species confirmation were done by MALDI-TOF MS and 16S rRNA sequencing. Strains were further characterized by single enzyme amplified fragments length polymorphism (SE-AFLP) and broth microdilution techniques.

\textbf{Results:} All three isolates were confirmed as \textit{G. sulfidifaciens}. The SE-AFLP genotyping resulted in distinct fingerprint patterns for each strain. All isolates presented high MIC values to tetracycline, sulfonamides, aminoglycosides and tyllosin tartrate, which present high usage in human and animal medicine.

\textbf{Conclusions:} \textit{Globicatella sulfidifaciens} could be related to sporadic urinary tract infections in swine and appear to present alarming antimicrobial susceptibility profile. It is necessary to differentiate \textit{Streptococcus}-like microorganisms in routine laboratory diagnostics for the correct identification of underestimated species potentially pathogenic to animals.

1. Introduction

The \textit{Globicatella} genus comprises Gram-positive, facultative anaerobic, \(\alpha\)-hemolytic and catalase negative cocci (Vandamme et al. 2001). These bacteria are morphologically and phenotypically very similar to \textit{Streptococcus} and \textit{Aerococcus} genus which can lead to misidentification of the pathogen and, consequently, the underestimation of \textit{Globicatella} infections. However, they can be genotypically differentiated (Lau et al. 2006; Héry-Arnaud et al. 2010).

\textit{Globicatella} species have already been isolated from human and animals with heart and brain disorders, as well as urinary tract infection. \textit{Globicatella sulfidifaciens} has been described in pulmonary and articular exudates from various animal species, including bovine, swine and ovine (Vandamme et al. 2001), while \textit{G. sanguinis} has been related to human septicemia, meningitis, endocarditis and urinary tract infection (Shewmaker et al. 2001; Seegmüller et al. 2007; Héry-Arnaud et al. 2010; Matsunami et al. 2012). Here we report the isolation, identification and molecular and antibiotic susceptibility characterization of \textit{G. sulfidifaciens} from swine urinary tract infection.

2. Material and methods

Urine samples from 140 sows of two swine herds located in São Paulo State (Brazil) yielded the isolation of three presumptive \textit{Globicatella} strains (U16, U17, U90). Both herds were presented with a history of recurrent urinary tract infection. Sows midstream urine samples were taken using sterile universal sample collector after spontaneous micturition in the first hour of morning. All urine samples presented characteristics suggestive of urinary tract infection based on dipstick test screening results (leukocyturia, nitrite presence, proteinuria and pH > 7.5).

Briefly, selected urine samples (10 mL) were centrifuged at 4,000 \(x\) g for 10 min and the obtained pellet was plated on blood agar (5% defibrinated sheep blood). The \textit{Streptococcus}-like colonies were initially screened by matrix-assisted laser desorption
ionization–time of flight mass spectrometry (MALDI-TOF MS) identification. Mass spectra were acquired using a Microflex™ mass spectrometer (Bruker Daltonik, Bremen, Germany) and identified with manufacturer’s software MALDI BioTyper™ 3.0.

For the species confirmation, 16S rRNA gene amplification was performed using primers according to Twomey et al. (2012). The 1370 bp amplified fragments were purified using Illustra GFX™ PCR DNA and Gel Band Purification kit (GE Healthcare, Piscataway, USA) and sequenced at the Human Genome Research Center (University of Sao Paulo, Brazil). The phylogenetic analysis was performed with Mega 5.10 software (Tamura et al. 2011) using the maximum-likelihood method and 500 bootstrap replicates for branch support statistical inference.

*Globicatella sulfidifaciens* strains were further genotyped by single enzyme amplified fragments length polymorphism (SE-AFLP) following McLauchlin et al. (2000) protocol. Fingerprint patterns were analyzed by comprehensive pairwise comparisons using Dice coefficient. A dendrogram was generated by Bionumerics 7.5 (Applied Maths, Saint-Martens-Latem, Belgium) and a 90% genetic similarity cut-off value was applied for cluster analysis (van Belkum et al. 2007).

The minimal inhibitory concentration (MIC) was determined by broth microdilution technique (CLSI [Clinical and Laboratory Standards Institute] 2013) using Sensititre® Standard Susceptibility MIC Plate (TREK Diagnostic Systems/Thermo Fisher Scientific, Waltham, USA). *Streptococcus pneumoniae* ATCC 49619 was used as internal control.

### 3. Results

Only three of the selected urine samples were positive for *G. sulfidifaciens* according to the MALDI-TOF MS screening with log (score) values > 2.0. The 16S rRNA sequencing confirmed the MALDI-TOF MS identification clustering our isolates with *G. sulfidifaciens* (Figure 1). The DNA sequences from this study were deposited in GenBank under accession numbers KT825513–KT825515. However, the 16S rRNA analysis enabled *Globicatella* species differentiation by only 0.3% nucleotide difference. The strains were also tested using the Vitek® system (Gram-positive identification; GPI) (bioMérieux, Hazelwood, USA) by which all three strains were confirmed as *G. sulfidifaciens* with > 98% of confidence.

All *G. sulfidifaciens* isolates clustered separately in SE-AFLP (Figure 2) despite the higher genetic similarity between U16 and U90 strains. The U17 strain presented a distinct fingerprint pattern from the other studied strains, even though it originated from the same herd of the U16 isolate.

![Figure 1](image-url)  
**Figure 1.** Phylogenetic tree based on the 16S rRNA nucleotide sequences for *Globicatella* species confirmation. The bootstrap values are presented at the corresponding branches.
The MIC values of the studied isolates are presented in Table 1. All isolates presented high MIC values to tetracycline, sulphonamides, aminoglycosides and tylosin. The enrofloxacin and the tested β-lactams presented the smallest MIC values and variation observed for the three strains.

4. Discussion

The complexity for species differentiation by 16S rRNA gene sequencing was expected since Lau et al. (2006) had already reported low nucleotide divergence between Globicatella species. These findings corroborate that 16S rRNA sequence analysis alone is not a reliable tool for Globicatella species identification (Lau et al. 2006; Héry-Arnaud et al. 2010). Therefore, to confirm the species identification, the strains were also tested using the Vitek® system.

Globicatella genotypic characterization has been applied only once before with pulsed field gel electrophoresis (PFGE). Héry-Arnaud et al. (2010) reported that PFGE could discriminate Globicatella species and even highlighted differences in the G. sanguinis invasive isolates band patterns compared to rectal isolate and type strains. The proposed SE-AFLP also appears to be highly discriminatory at least for Globicatella intra-species analysis. However, further strains should be analyzed to corroborate the value of this technique for Globicatella study.

Our results revealed an alarming susceptibility profile for porcine Globicatella isolates which presented high MIC values for antimicrobials with high usage in both animal and human medicine. Also, the U90 strain stands out with higher MIC values also to fluoroquinolone, erythromycin and clindamycin, similarly to human Globicatella invasive isolates (Héry-Arnaud et al. 2010; Matsunami et al. 2012).

Even though G. sulfidifaciens still is not considered a major concern for swine health, there is the risk that they could transfer their resistance genes to other Streptococcus-like microorganisms and further disseminate antimicrobial resistance for the normal flora, as previously observed for streptococci (Seppälä et al. 2003).

In summary, G. sulfidifaciens could be related to sporadic urinary tract infections in swine. The clinical relevance of G. sulfidifaciens in animals and its zoonotic potential remains unknown due to the difficulty in its identification. Therefore, the necessity to differentiate Streptococcus-like microorganisms in routine laboratory diagnostics increases significantly with the description of new cases of these underestimated pathogens.

Acknowledgments

Carlos E. C. Matajira, Vasco T. M. Gomes and Luisa Z. Moreno are recipients of FAPESP PhD fellowships. Andrea M. Moreno is a CNPq fellow.

Disclosure statement

No conflict of interest was reported by the authors.

Funding

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) [grant number 2015/26159-1], Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) [grant number 2013/16946-0] and Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) [grant number 2013/17136-2].

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Figure 2. Dendrogram showing the relationship among the SE-AFLP patterns of porcine Globicatella sulfidifaciens strains.

| Antimicrobial          | Range (MIC µg/mL) | U16 | U17 | U90 |
|------------------------|-------------------|-----|-----|-----|
| Penicillin             | 0.06–8.0          | <0.06 | 0.25 | 1.0 |
| Amoxicillin            | 0.25–16.0         | <0.25 | 0.25 | <0.25 |
| Cefoxitin              | 0.25–4.0          | 1.0 | 1.0 | 0.5 |
| Enrofloxacin           | 0.12–2.0          | 0.5 | 1.0 | 2.0 |
| Gentamicin             | 0.5–8.0           | <0.5 | 0.5 | 4.0 |
| Erythromycin           | 0.12–4.0          | <0.12 | <0.12 | >4.0 |
| Neomycin               | 2.0–32.0          | 32.0 | 16.0 | >2.0 |
| Streptomycin           | 8.0–1024.0        | 64.0 | 64.0 | 64.0 |
| Spectinomycin          | 8.0–64.0          | >64.0 | >64.0 | >64.0 |
| Oxytetracycline        | 0.25–8.0          | >8.0 | 8.0 | 4.0 |
| Tetracycline           | 0.25–8.0          | >8.0 | 8.0 | 8.0 |
| Sulphathiazole         | 32.0–256.0        | >256.0 | >256.0 | >256.0 |
| Sulphadimethoxine      | 32.0–256.0        | >256.0 | >256.0 | >256.0 |
| Trimethoprim /         | 0.5/0.9–2/38      | >2/38 | >2/38 | >2/38 |
| sulfamethoxazole       |                   |      |     |     |
| Clindamycin            | 0.5–4.0           | <0.5 | <0.5 | >4.0 |
| Tylosin tartrate       | 2.5–20.0          | 10.0 | 10.0 | >20.0 |
| Florfenicol            | 1.0–8.0           | <1.0 | <1.0 | 4.0 |
| Novobiocin             | 0.5–4.0           | <0.5 | >4.0 | <0.5 |
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