



Short communication

Molecular and antibiotic susceptibility characterization of *Aerococcus viridans* isolated from porcine urinary infection

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ABSTRACT

Aerococcus viridans has been reported as a human and animal pathogen causing urinary tract infection, arthritis, pneumonia, meningitis and endocarditis. Routinely, *A. viridans* is not surveyed in clinical diagnosis laboratories and commonly is misidentified as other bacteria. There is no concrete data on the prevalence and impact of the pathogen to both human and animal health. In the present study, we report the isolation and molecular and antibiotic susceptibility characterization of *A. viridans* strains from porcine urinary infections. A total of 22 isolates were identified as *A. viridans* by MALDI-TOF MS and confirmed by 16S rRNA gene sequencing. Isolates were genotyped by single enzyme amplified fragments length polymorphism (SE-AFLP) that resulted in 19 clusters of which 81.2% were composed by single isolates. The high genetic heterogeneity corroborates previous studies and appears to be a particularity of *A. viridans*. The minimal inhibitory concentration (MIC) values also presented variability especially for ceftiofur, fluoroquinolones and aminoglycosides. The high MICs of aminoglycosides, tetracyclines and macrolides seen among the *A. viridans* corroborate previous reports and the widespread veterinary usage of these antibiotics demand attention for the implication of *A. viridans* infection to both human and animal health.

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1. Introduction

The *Aerococcus* genus is composed by gram positive cocci that are facultative anaerobic, oxidase and catalase negative (Williams et al., 1953). These bacteria are phenotypically similar to staphylococci and streptococci which can cause misidentification of the pathogen and, consequently, the underestimation of aerococcal infections (Rasmussen, 2012).

Aerococcus viridans was the first species described among the genus and has been reported as a human pathogen causing urinary tract infection, arthritis and endocarditis (Razeq et al., 1999;

Gopalachar et al., 2004; Popescu et al., 2005). In clinical veterinary practice, *A. viridans* has been associated with bovine mastitis (Devriese et al., 1999; Liu et al., 2015) and also as the causative agent of arthritis, pneumonia and meningitis in swine (Martin et al., 2007).

There is no concrete data on the prevalence and impact of the pathogen to both human and animal health. Other *Aerococcus* species became more described by the introduction of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) as a rapid and accurate method for microbial identification (Rasmussen, 2012; Senneby et al., 2013).

The few studies, which addressed the *A. viridans* molecular characterization, high genetic heterogeneity was observed independently of the applied technique (Martin et al., 2007; Liu et al., 2015). Variation between antimicrobial susceptibility profiles has also been reported among clinical isolates (Popescu et al., 2005; Martin et al., 2007; Rasmussen, 2012; Liu et al., 2015). Here we present the isolation, molecular and antibiotic susceptibility characterization of *A. viridans* from porcine urinary infection.

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2. Materials and methods

2.1. Samples and bacterial isolation

Sow urine samples from three swine herds located in São Paulo State (Brazil) yielded the isolation of 22 presumptive *Aerococcus* strains which were submitted to further tests. The urine samples suggestive of urinary tract infection were selected based on dipstick test screening results (leukocyturia, nitrite presence, proteinuria and pH > 7.5).

Briefly, urine samples (10 mL) were centrifuged at $4000 \times g$ for 10 min and the obtained pellet was plated on blood agar (5% defibrinated sheep blood). Isolates were maintained in a stock medium containing glycerol at -80°C and later reactivated in brain-heart infusion (BHI) medium (Difco, Sparks, MD, USA) supplemented with fetal calf serum (5%) for 24 h at 37°C and plated on blood agar (5% defibrinated sheep blood), under microaerophilic conditions, to assure purity.

2.2. MALDI-TOF MS bacterial identification

For the MALDI-TOF MS identification, bacterial proteins were extracted using an ethanol/formic acid protocol (Hijazin et al., 2012). The protein suspension (1 μL) was transferred to a polished steel target plate and allowed to dry at room temperature. The sample was overlaid with 1 μL of matrix (10 mg α -cyano-4-hydroxy-cinnamic acid mL^{-1} in 50% acetonitrile/2.5% trifluoroacetic acid), and mass spectra were acquired using a MicroflexTM mass spectrometer (Bruker Daltonik). The spectra were loaded into MALDI BioTyperTM 3.0 and compared with the patented manufacturer's library, which resulted in the log (score) value. Standard Bruker interpretative criteria were applied; scores ≥ 2.0 were accepted for species assignment and scores ≥ 1.7 but ≤ 2.0 for genus identification.

2.3. Species confirmation by 16S rRNA sequencing

For the confirmation of MALDI-TOF MS identification, representative isolates were selected for 16S rRNA sequencing. The partial amplification of the 16S rRNA gene was performed using Twomey et al. (2012) primers. Amplified fragments were purified using the Illustra GFXTM PCR DNA and Gel Band Purification kit (GE Healthcare) and sequenced directly by Human Genome Research

Center (University of Sao Paulo, Brazil). The BIOEDIT Sequence Alignment Editor 7.0.9 (Hall, 1999) was used for sequence editing and comparison with GenBank database was performed with the BLAST program. The DNA sequences from this study were deposited in GenBank under accession numbers KR819482–KR819484.

2.4. SE-AFLP genotyping

Single enzyme amplified fragments length polymorphism (SE-AFLP) was applied for *A. viridans* genotyping. The protocol of McLauchlin et al. (2000) was used for SE-AFLP and DNA fragments were detected through electrophoresis at 24V for 26 h in 2% agarose gel stained with BlueGreen[®] (LGC Biotecnologia, São Paulo, Brazil). SE-AFLP fingerprints were analyzed using the Dice coefficient by means of Bionumerics 7.5 software (Applied Maths NV, Saint-Martens-Latem, Belgium) to generate the dendrogram. A cut-off value of 90% of genetic similarity was applied to analyze the resulted clusters (Van Belkum et al., 2007).

2.5. Antibiotic susceptibility profiling

The minimal inhibitory concentration (MIC) was determined by broth microdilution technique as recommended by the Clinical and Laboratory Standards Institute for fastidious organisms (VET01-A4, 2013) using Sensititre[®] Standard Susceptibility MIC Plates BOPO6F (TREK Diagnostic Systems/Thermo Fisher Scientific, Waltham, MA, USA). *S. pneumoniae* ATCC 49619 and *S. aureus* ATCC 29213 were used as internal quality control. The MIC₅₀ and MIC₉₀ values for the respective antimicrobials were determined according to Schwarz et al. (2010).

3. Results and discussion

All 22 isolates were identified by MALDI BioTyperTM with log (score) values >2.0 for *Aerococcus viridans*. The partial 16S rRNA gene sequencing was performed on isolates U45, U62 and U83; sequences presented >99% identity with *A. viridans* ATCC 11563 (NZ_ADNT01000041). The MALDI-TOF MS proved to be a reliable technique for *A. viridans* diagnosis, as it has already been demonstrated for other genus of the *Streptococcaceae* family (Cherkaoui et al., 2011; Kudirkiene et al., 2015). Thus our data corroborate previous reports that MALDI-TOF MS is a reliable

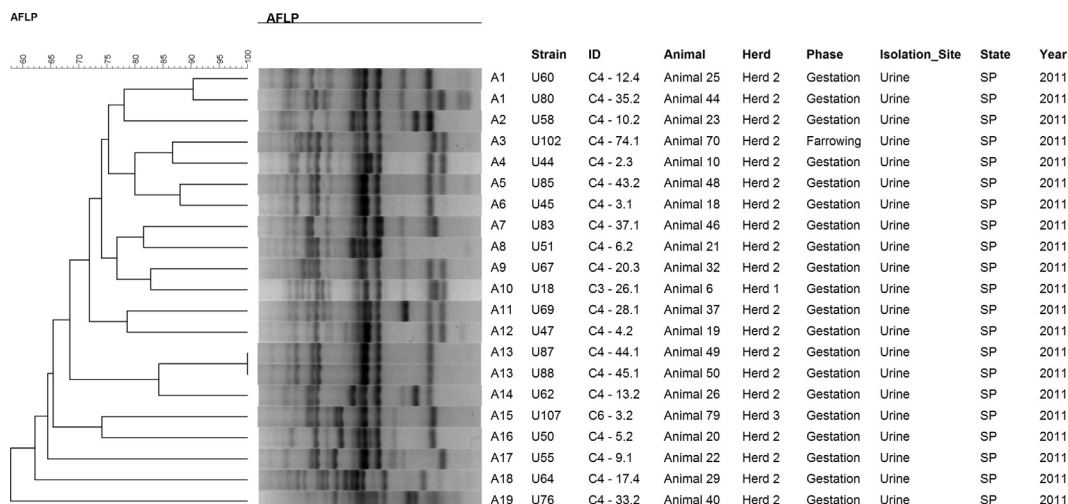


Fig. 1. Dendrogram showing the relationship among the SE-AFLP patterns from porcine *Aerococcus viridans* isolates.

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