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MICROBIOLOGY

Accurate identification of atypical *Staphylococcus chromogenes* plasma-clotting strains causing bovine mastitis

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ABSTRACT: *We compared the potential of routine techniques used for the identification of Staphylococcus species, aiming to evaluate their accuracy in the detection of 43 Staphylococcus chromogenes strains isolated from bovine mastitis that, despite being a coagulase-negative species, are able to clot plasma. These strains could be mistakenly suspected to be S. aureus and lead to an unappropriated treatment of the disease. MALDI-TOF, PCR-RFLP of the chaperonine gene groEL, and sequencing of the 16S rRNA and elongation factor Tu gene tuf were employed. Results from the four methods were coincident for only half of the strains because of the low accuracy of the groEL PCR-RFLP (51.2% accuracy). Even though all the sequencing results were identical, the high accuracy of the MALDI-TOF results (97.7% accuracy, with only one strain misidentified) encourage the use of this technique, since it does not require laborious sample preparation, being fast and simple to perform.*
Key words: *Staphylococcus chromogenes, mastitis, MALDI-TOF, tuf, groEL.*

Identificação acurada de cepas atípicas de *Staphylococcus chromogenes* que coagulam o plasma envolvidas com mastite bovina

RESUMO: *Nós comparamos o potencial de técnicas rotineiras utilizadas para a identificação de espécies de Staphylococcus, com o objetivo de avaliar a acurácia delas na detecção de 43 isolados de Staphylococcus chromogenes envolvidos com mastite bovina que, apesar de ser uma espécie coagulase-negativa, são capazes de coagular o plasma. Essas cepas poderiam ser erroneamente suspeitas de serem S. aureus e levarem a um tratamento não adequado da doença. MALDI-TOF, PCR-RFLP do gene da chaperonina groEL e sequenciamento do gene do rRNA 16S e do gene do fator de elongação Tu, tuf, foram avaliados. Os resultados dos quatro métodos foram coincidentes para apenas metade das cepas, devido à baixa precisão da PCR-RFLP com groEL (51,2% de acurácia). Apesar de todos os resultados do sequenciamento serem idênticos, a alta precisão dos resultados do MALDI-TOF (97,7% de acurácia, com apenas uma cepa identificada incorretamente) encoraja o uso dessa técnica, pois, não requer preparação laboriosa de amostras, sendo rápida e simples de executar.*
Palavras-chave: *Staphylococcus chromogenes, mastite, MALDI-TOF, tuf, groEL.*

Staphylococcus aureus, the major pathogen from its genus, causes a wide range of clinical infections in human beings and animals (TONG et al., 2015). Discrimination between *S. aureus* from most staphylococci is primarily made by evaluating its ability to produce the enzyme coagulase, which promotes clotting of plasma. This activity is the foundation of routine identification tests and helps in the differentiation between *S. aureus* and coagulase-negative staphylococci (CoNS), which were long considered as harmless environmental contaminants, but are emerging as important opportunist pathogens (BECKER et al., 2014). However, our group has recently described *Staphylococcus chromogenes* strains, isolated from dairy animals with mastitis, that are surprisingly capable of clotting plasma, besides being a coagulase-negative species (SANTOS et al., 2016).

Among CoNS, *Staphylococcus chromogenes* is one of the major pathogens involved with mastitis in dairy animals (VANDERHAEGHEN et al., 2014), while *S. aureus* is the major coagulase-positive pathogen causing the same disease (BARKEMA et al., 2006). For a long time overlooked, *S. chromogenes* has also been isolated from hospitals and other healthcare facilities (CATANO et al., 2012; ZEMOURI et al., 2017; ZHENZHEN et al., 2018), in addition to being shown colonizing HIV-positive patients (BACK-BRITO et al., 2011), and causing bloodstream infections in patients with AIDS (ADEYEMI et al., 2010). Then, the coagulase-

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positive phenotype of *S. chromogenes* shown by our group can easily lead to misidentification and inappropriate treatment of the disease caused by them.

Aiming to search for accurate methods to identify strains showing atypical results in the coagulation assays, we estimated the identification potential of four different techniques: Matrix-Assisted Laser Desorption Ionization-Times of Flight Mass Spectometry (MALDI-TOF), PCR-restriction fragment length polymorphism (PCR-RFLP) of the chaperonine gene *groEL*, and DNA sequencing of the 16S rRNA and elongation factor Tu gene *tuf*.

The *S. chromogenes* strains (n=43) studied in our previous research (SANTOS et al., 2016), were first analyzed in triplicate by MALDI-TOF as described before (TOMAZI et al., 2014). Mass spectral data were collected within the m/z range of 2,000 to 20,000, and the data were acquired and analyzed using the FlexControl software 3.3 (Bruker Daltonics, MA, USA). A PCR-RFLP of the *groEL* gene strategy, standardized by our group to differentiate several relevant *Staphylococcus* species (SANTOS et al., 2008), was then performed. The identification data obtained were compared to those from the previous DNA sequencing of the 16S rRNA and *tuf* genes (SANTOS et al., 2016).

Only 23 (51.2%) strains displayed coincident results for all the four methods (Figure 1), indicating strain variation, as previously observed (SANTOS et al., 2016). However, because all results from DNA sequencing were identical, all strains were confirmed as being *S. chromogenes*. All strains had MALDI-TOF scores≥2.3, indicating reliable identification of the species. The *groEL* PCR-RFLP was the less accurate (51.2% accuracy) method, as 21 (48.8%) strains were either identified as *Staphylococcus caprae* (n=20) or undetermined. The MALDI-TOF had 97.7% accuracy, with only one strain misidentified. This result is consistent with the high accuracy of the technique reported for several *Staphylococcus* species obtained from different origins, such as clinical, food samples and plants (DUBOI et al., 2010), domestic dogs (SILVA et al., 2015) and domestic cats (ROSSI et al., 2017).

For being faster, cost-effective and easier to perform, allowing diagnosis using intact cells or cell extracts, this technique is an emergent identification method (ZHU et al., 2015). The equipment is available in most major university centers and many large hospitals in Brazil and the cost per analysis of each sample is less than fifty cents of dollar. In addition, the MALDI-TOF analysis service is offered by international companies, such as MIDI Labs (USA, http://midilabs.com) and BIOTECON Diagnostics (Germany, http://www.bc-diagnostics.com).

Our results, together with the aforementioned advantages, encourage the use

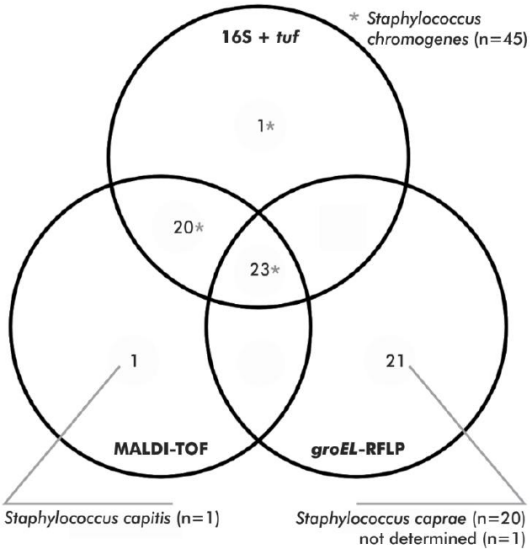

Figure 1 - Comparison of results obtained from four methods (MALDI-TOF, groEL-RFLP and sequencing of *tuf* and 16S rRNA genes) used for the identification of 43 coagulase-negative *Staphylococcus* strains showing atypical results in rabbit plasma coagulation assays.

of MALDI-TOF as the identification method for *Staphylococcus* species isolated from cows with mastitis even for atypical *S. chromogenes* strains that are able to clot plasma and are circulating in Brazilian farms.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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