Staphylococcus chromogenes, a Coagulase-Negative *Staphylococcus* Species That Can Clot Plasma

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Staphylococcus chromogenes is one of the main coagulase-negative staphylococci isolated from mastitis of dairy cows. We describe *S. chromogenes* isolates that can clot plasma. Since the main pathogen causing mastitis is coagulase-positive *Staphylococcus aureus*, the coagulase-positive phenotype of *S. chromogenes* described here can easily lead to misidentification.

Among the group of coagulase-negative staphylococci (CoNS) commonly isolated from dairy cow mastitis, *Staphylococcus chromogenes* is one of the most prevalent (1). However, routine mastitis diagnostics treat CoNS as a uniform group, even in view of the finding that some species are more harmful to the mammary gland than others (1).

The traditional approach in most clinical laboratories for the differentiation of CoNS and coagulase-positive *Staphylococcus* (CoPS) is the tube coagulase test, which is based on the ability of an extracellular staphylococcal coagulase to clot plasma by converting fibrinogen to fibrin (2). Studies on the coagulase factor (Coa) date back more than 100 years. Virtually all *Staphylococcus aureus* isolates secrete Coa (3–5), together with a second coagulase, designated von Willebrand factor-binding protein (vWbp), which catalyzes a reaction similar to that of Coa (6). Coa and vWbp are the main factors known to be capable of converting fibrinogen to fibrin and, thus, generating clots (7). Other *Staphylococcus* spp. factors, such as the clumping factor Efb (extracellular fibrinogen binding protein) and Eap (extracellular adherence protein), can also bind fibrinogen or fibrin, but they do not cause clotting (8, 9).

In this report, we describe 42 *S. chromogenes* isolates that were sent to our laboratory with ambiguous identification. These isolates were obtained between March and November 2003 from milk samples of buffalo with subclinical mastitis and kept in the culture collection at the Embrapa Dairy Cattle Research Center (Juiz de Fora, MG, Brazil). Strains were considered causative of intramammary infection when present in pure cultures of six or more colonies on ovine blood agar plates. These strains were initially classified as CoPS by the coagulase test (Coagu-Plasma, Laborclin, Pinhais, PR, Brazil) but later identified as *S. chromogenes* using the 16S rRNA gene (similarity ≥99%; accession number AY688044.1). Since *S. chromogenes* belongs in the coagulase-negative group, the phenotypical trait of coagulase positivity has so far been unreported in this species.

To confirm the species, all 42 isolates were submitted to a new round of identification using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), as described by Tomazi et al. (10), and *tuf* gene sequencing (similarity ≥97%; accession number HM352952.1), as described by Hwang et al. (11). Sequencing of the *tuf* gene and MALDI-TOF MS confirmed that these isolates were *S. chromogenes*, with 100% agreement with the identification previously obtained by se-

FIG 1 Clot formation in rabbit plasma. Coagulase test using rabbit plasma after 24 h of incubation with *S. aureus* ATCC 12600 (A), *S. chromogenes* clotting plasma (B), and coagulase-negative *S. epidermidis* (C).

quencing of the 16S rRNA gene. The species identification results are consistent with those reported by Lange et al. (12) and Tomazi et al. (10), who successfully identified *S. chromogenes* from bovine mastitis by use of these methodologies.

To determine the coagulase activity of the isolates, the coagulase test was reproduced in triplicate with a commercial kit (CP assay) (Coagu-Plasma, Laborclin, Pinhais, PR, Brazil), following the recommendations of the manufacturer, and once using rabbit plasma *in natura* (RP assay) (Pastorex Staph Plus, Bio-Rad), as described by Sperber and Tatini (13). Figure 1 shows the clot

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FIG 2 Genetic relationship of the isolates. Cladogram generated by GelCompare software. Scales at top and bottom on the left show similarity. Dashed line, 80% similarity cutoff points. Center, gel photos with bands marked (PFGE). Isolate, information of the isolates index; Type, the type assigned using the 80% threshold; Herd, herd from which each one was isolated; CP, the result of the Coagu-Plasma; RP, coagulation assay on rabbit plasma; CF, clumping factor assay; + and -, positive and negative results in the three assays; V, variable for isolates presenting discordant results (positive or negative) on the triplicate experiments for CP.

formed in the test by use of the RP assay. With the CP assay, 23 isolates were consistently positive and 8 were consistently negative in the triplicate assays (Fig. 2). The remaining 11 isolates presented at least one discordant result, which is most intriguing.

This may be due to the isolates producing a low level of the Coa or to coagulase with lower activity that was not detected by the level of sensitivity of the kit.

In the RP assay, all isolates that were classified as variable by the

CP assay yielded a negative reaction, with the exception of isolate 5294 (Fig. 2), which was found to be RP positive. Additionally, only two isolates presented discordant results with the two methodologies (5373 was CP⁻/RP⁺, 5207 was CP⁺/RP⁻).

The coagulase test was the first test used to classify staphylococci, and *S. chromogenes* is phylogenetically close to many coagulase-positive and coagulase-variable species (2). In the genome sequence of *S. chromogenes*, we found an open reading frame sharing 41% identity with the predicted coagulase gene of the coagulase-positive *Staphylococcus pseudintermedius* (14). This finding reinforces the need for identification at the species level and inclusion of molecular protocols for species determination.

Furthermore, to evaluate genetic diversity, we performed genotyping by pulsed-field gel electrophoresis (PFGE) using SmaI (15). PFGE revealed a high diversity, which was expected since veterinary *Staphylococcus* spp. isolates reveal more phenotypic variability than human isolates (2). Using an 80% similarity threshold, we found 28 clusters by analysis of band patterns from 42 isolates obtained from 7 herds. As observed in Fig. 2, there seems to be some correlation between PFGE-type clustering and the presence or absence of a plasma-clotting phenotype. Indeed, five prevalent PFGE types (A, B, D, Q, and Y) comprised a total of 17 (74%) of the 23 CP⁺ isolates. It is well known for CoPS that the presence of coagulase is considered an indicator for pathogenic potential of the isolates (2). No correlation was observed between clotting ability by either method (CP or RF assay) and the clumping factor test.

Our observations showed that *S. chromogenes* can be a coagulase-variable *Staphylococcus* sp., with rare but individual populations displaying clotting activity. PFGE is a genomewide typing methodology commonly used for the genus *Staphylococcus* and permits the study of genetic relatedness among strains. However, in the light of our findings regarding the coagulase phenotyping in the *S. chromogenes* population, other analyses, such as multilocus sequence typing, are necessary to show phylogenetic relationships among the isolates.

Unrelated to the coagulase phenotype, but reinforcing the presence of atypical traits, 18 strains yielded a positive reaction in the clumping factor test. Ten of these were positive for both the clumping factor and Coa. The clumping factor is also heavily associated with *S. aureus* and its virulence (8, 9). The coagulase test is among the first tests used in the routine laboratory to identify and classify staphylococci and is often the only one utilized (2). However, the presence of variable phenotypes, such as those described here, can often lead to misidentification (16). In the case of *S. chromogenes*, no coagulase-positive strain has been reported, to our knowledge, but here we show a number of *S. chromogenes* strains capable of clotting plasma. Recently, Taponen et al. (16) reported a coagulase-variable *Staphylococcus*, namely, *Staphylococcus agnetis*, a new species isolated from bovine mastitis. In fact, according to Becker et al. (2), *Staphylococcus hyicus* is also a coagulase-variable *Staphylococcus* species. *S. agnetis* and *S. hyicus* are closely related to *S. chromogenes*, but so far, nothing regarding the ability to clot plasma has been mentioned for *S. chromogenes*.

In conclusion, we report isolates of coagulase-negative *S. chromogenes* that have the ability to clot plasma, presenting an atypical phenotype related to this species. Further studies are required to elucidate the mechanisms that confer the ability to clot plasma to these *S. chromogenes* isolates and the role of this phenotype in the

virulence of this species. In addition, we are aware that this particular phenotype may easily lead to misidentification with the pathogen *S. aureus*. Therefore, the remaining question is whether the coagulase-negative *S. chromogenes* should be reclassified as a coagulase-variable species.

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REFERENCES

- Vanderhaeghen W, Piepers S, Leroy F, Van Coillie E, Haesebrouck F, De Vliegher S. 2014. Effect, persistence, and virulence of coagulase-negative *Staphylococcus* species associated with ruminant udder health. *J Dairy Sci* 97:5275–5293. <http://dx.doi.org/10.3168/jds.2013-7775>.
- Becker K, Heilmann C, Peters G. 2014. Coagulase-negative staphylococci. *Clin Microbiol Rev* 27:870–926. <http://dx.doi.org/10.1128/CMR.00109-13>.
- Field HI, Smith HW. 1944. Coagulase test for staphylococci. *J Comp Pathol* 55:63–69.
- Loeb L. 1903. The influence of certain bacteria on the coagulation of blood. *J Med Res* 10:407–419.
- Smith W, Hale JH, Smith MM. 1947. The role of coagulase in staphylococcal infections. *Br J Exp Pathol* 28:57–67.
- Bjerketorp J, Jacobsson K, Frykberg L. 2004. The von Willebrand factor-binding protein (vWbp) of *Staphylococcus aureus* is a coagulase. *FEMS Microbiol Lett* 234:309–314. <http://dx.doi.org/10.1111/j.1574-6968.2004.tb09549.x>.
- Cheng AG, McAdow M, Kim HK, Bae T, Missiakas DM, Schneewind O. 2010. Contribution of coagulases towards *Staphylococcus aureus* disease and protective immunity. *PLoS Pathog* 6:e1001036. <http://dx.doi.org/10.1371/journal.ppat.1001036>.
- Geoghegan JA, Ganesh VK, Smeds E, Liang X, Hook M, Foster TJ. 2010. Molecular characterization of the interaction of staphylococcal MSCRAMMs ClfA and Fbl with fibrinogen. *J Biol Chem* 285:6208–6216. <http://dx.doi.org/10.1074/jbc.M109.062208>.
- Heilmann C, Herrmann M, Kehrel BE, Peters G. 2002. Platelet-binding domains in 2 fibrinogen-binding proteins of *Staphylococcus aureus* identified by phage display. *J Infect Dis* 186:32–39. <http://dx.doi.org/10.1086/341081>.
- Tomazi T, Gonçalves JL, Barreiro JR, de Campos Braga PA, Silva LFP, Eberlin MN, dos Santos MV. 2014. Identification of coagulase-negative staphylococci from bovine intramammary infection by matrix-assisted laser desorption/ionization–time of flight mass spectrometry. *J Clin Microbiol* 52:1658–1663. <http://dx.doi.org/10.1128/JCM.03032-13>.
- Hwang SM, Kim MS, Park KU, Song J, Kim EC. 2011. *tuf* gene sequence analysis has greater discriminatory power than 16S rRNA sequence analysis in identification of clinical isolates of coagulase-negative staphylococci. *J Clin Microbiol* 49:4142–4149. <http://dx.doi.org/10.1128/JCM.05213-11>.
- Lange CC, Brito MA, Reis DR, Machado MA, Guimarães AS, Azevedo AL, Meurer IR. 2015. Species-level identification of staphylococci isolated from bovine mastitis in Brazil using partial 16S rRNA sequencing. *Vet Microbiol* 176:382–388. <http://dx.doi.org/10.1016/j.vetmic.2015.01.024>.
- Sperber WH, Tatini SR. 1975. Interpretation of the coagulase test tube for identification of *Staphylococcus aureus*. *Appl Microbiol* 29:502–505.

14. Fry PR, Calcutt MR, Foecking MF, Hsleh HY, Suntrup DG, Perry J, Stewart GC, Middleton JR. 2014. Draft of genome sequence of *Staphylococcus chromogenes* strain MU 970, isolated from a case of chronic bovine mastitis. *Genome Announc* 2:e00835–14. <http://dx.doi.org/10.1128/genomeA.00835-14>.
15. Nunes AP, Teixeira LM, Bastos CC, Silva MG, Ferreira RB, Fonseca LS, Santos KRN. 2005. Genomic characterization of oxacillin-resistant *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* isolated from Brazilian medical centers. *J Hosp Infect* 59:19–26. <http://dx.doi.org/10.1016/j.jhin.2004.07.021>.
16. Taponen S, Supré K, Piessens V, Van Coillie E, De Vlieghe S, Koort JMK. 2012. *Staphylococcus agnetis* sp. nov., a coagulase-variable species from bovine subclinical and mild clinical mastitis. *Int J Syst Evol Microbiol* 62:61–65. <http://dx.doi.org/10.1099/ijs.0.028365-0>.