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Short communication: Characterization of methicillin-resistant *Staphylococcus aureus* isolated from raw milk fresh cheese in Colombia

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ABSTRACT

The aim of this study was the characterization of a collection of 8 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, obtained from samples of fresh cheese (Doble Crema) elaborated from raw cow milk in small dairies in Colombia. All the isolates harbored the *mecA* and Panton-Valentine leukocidin (PVL) genes, presented with SCC*mec* type IV, and belonged to multilocus sequence type 8 and *spa* type 024. Seven isolates presented 3 closely related pulsed-field gel electrophoresis profiles. Three of them carried the staphylococcal enterotoxin B gene. The isolates were resistant to cefoxitin, oxacillin, penicillin, and ampicillin and susceptible to all non-β-lactams antibiotics tested, with minimum inhibitory concentration values for oxacillin of 4 to 8 mg/L. The isolates belonged to the community-acquired MRSA group, suggesting a human source of contamination. The risk of human infection by MRSA via contaminated foods is considered low, but contaminated food commodities can contribute to the worldwide dissemination of clones of community-acquired MRSA.

Key words: methicillin-resistant *Staphylococcus aureus*, MRSA, Doble Crema cheese

Short Communication

Methicillin-resistant *Staphylococcus aureus* (**MRSA**) is a microorganism of concern as an important cause of nosocomial infections (hospital-associated MRSA). In recent years, the appearance of cases outside the hospital environment has put the focus in the so-called community-associated or community-acquired MRSA strains (**CA-MRSA**). The CA-MRSA are considered a serious threat to public health because they can be easily spread through healthy carriers and they present additional virulence factors that make them more likely to cause disease (Weber, 2005). The CA-MRSA strains generally carry the Panton-Valentine leukocidin (**PVL**) gene, staphylococcal chromosomal cassette *mec* (**SCC*mec***) type IV, and are susceptible to antimicrobials other than β-lactams (Vandenesch et al., 2003). The third significant type of MRSA is associated with livestock animals, which was initially detected in swine farms in Europe and nowadays is distributed worldwide and has spread to humans and other animal species. Most livestock-associated MRSA isolates are resistant to tetracyclines and do not carry either the PVL gene or staphylococcal enterotoxin (**SE**) genes (EFSA, 2009; Doyle et al., 2011).

Even though the isolation of MRSA from livestock and foods of animal origin has been reported extensively, the effect of MRSA in food-related illness is very low. To date, only 2 outbreaks of MRSA involving food have been reported (Kluytmans et al., 1995; Jones et al., 2002). However, there are some concerns whether food can constitute a reservoir of MRSA and food handlers can become infected with MRSA via food manipulation (EFSA, 2009; Doyle et al., 2011).

The prevalence of MRSA in cattle appears to be low (Alves et al., 2009), but there are reports of isolation of MRSA as an agent of intramammary infection (Lee, 2006; Luini et al., 2015) and also from bulk tank milk and dairy products (Normanno et al., 2007; Virgin et al., 2009; Can and Çelik, 2012). Strains of MRSA are disseminated worldwide and *S. aureus* remains as an important foodborne pathogen in Latin America (Pires et al., 2012); however, studies on the prevalence of MRSA in food commodities in Latin America are scarce (Rizek et al., 2011; Vanegas López et al., 2012). In a study carried out in Colombia by Vanegas-López et al. (2012), they identified 5 MRSA strains (one of them from milk cream) among 149 isolates.

Doble Crema (double cream) cheese is one of the most popular cheeses in Colombia, accounting for more than 30% of total cheese consumption. It is a fresh cheese similar to mozzarella and other stretched curd

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cheeses and traditionally made from raw cow milk in small dairies in Colombia. In research carried out on Doble Crema cheese, we characterized 65 isolates of coagulase-positive staphylococci and 18.5% of them were MRSA carrying the *mecA* gene (Herrera and Santos, 2015a,b). The aim of this study was to characterize the collection of 8 MRSA isolates by genetic typing and antimicrobial susceptibility testing.

The isolates were obtained from samples of raw milk cheese (Doble Crema cheese) collected at the retail level between April 2012 and April 2013 and identified as coagulase-positive staphylococci and MRSA as previously described (Herrera and Santos, 2015a,b). Briefly, *S. aureus* counts were determined on Baird Parker Agar with egg yolk-tellurite (Oxoid, Basingstoke, UK) and up to 3 typical colonies were confirmed as coagulase-positive by coagulase test (Lancette and Bennet, 2001). Isolates from 8 cheese samples carried the *mecA* gene and were considered as MRSA. Bacterial strains were preserved at −80°C in Brain Heart Infusion (BHI; Oxoid) broth plus 30% glycerol and routinely cultured in BHI broth at 37°C.

Antimicrobial susceptibility testing of the isolates was performed using the disk diffusion method recommended by EUCAST (www.eucast.org) against the following antimicrobials: cefoxitin, oxacillin, penicillin, ampicillin, gentamicin, ciprofloxacin, levofloxacin, norfloxacin, ofloxacin, teicoplanin, vancomycin, clindamycin, azithromycin, clarithromycin, erythromycin, chloramphenicol, nitrofurantoin, rifampicin, trimethoprim-sulfamethoxazole, trimethoprim, and tetracycline, using *S. aureus* CECT 794 as the control strain. Isolates were classified as susceptible or resistant according to the EUCAST breakpoint Table 2016 v6.0. Minimum inhibitory concentration values for oxacillin were determined with M.I.C. Evaluator strips (Oxoid) according to the manufacturer's instructions.

Amplification of genes responsible for production of selected enterotoxins (SEA, SEB, SEC, SED, SEE, SEG, SEH, and SEI), resistance to methicillin (*mecA* and *mecC*), production of PVL (*lukS/F*), and the presence of the *arc*A gene of the arginine catabolic mobile element (**ACME**) was carried out from a fresh culture of each isolate. An aliquot of 1 mL was centrifuged and the pellet was treated with 200 μL of InstaGene matrix (Bio-Rad, Hercules, CA) to release the DNA. Primers and conditions of the PCR protocols were described elsewhere (Lina et al., 1999; Smyth et al., 2001; Jarraud et al., 2002; Diep et al., 2008; García-Álvarez et al., 2011).

The SCC*mec* type was determined by the multiplex-PCR procedure described by Boye et al. (2007). The *spa* type was established by amplifying and sequencing the *spa* gene (Shopsin et al., 1999) and sequences were analyzed with spaTyper (http://spatyper.fortinbras.us/). Pulsed-field gel electrophoresis (**PFGE**) was carried out as described by McDougal et al. (2003). Comparison of profiles was done with the GelCompar 6.5 software (Applied Maths, St. Martens Latem, Belgium). Similarities were obtained using the Dice coefficient at 0.5% optimization and 1.25% tolerance, and a dendrogram was constructed with the unweighted-pair group method using the arithmetic mean clustering method. Multilocus sequence typing was performed following the procedure of Enright et al. (2000), and the allele number and sequence type of the isolates were assigned by comparison with data available in the multilocus sequence typing database (http://saureus.beta.mlst.net/).

The 8 isolates were resistant to cefoxitin (30 μg), oxacillin (1 μg), and the β-lactam antibiotics and susceptible to all non-β-lactam antibiotics tested. The MIC values for oxacillin were 4 mg/L for 6 isolates (FH 30, FH 32, FH 34, FH 44, FH 65, and FH 67) and 8 mg/L for 2 isolates (FH 38 and FH 61), which are values that allow classification of isolates as MRSA. All the isolates harbored the *mecA* and the PVL *luk*S/*luk*F genes, which are characteristic features of the CA-MRSA group (Vandenesch et al., 2003). The *mecC* gene was not detected in any MRSA isolate; this homolog of *mecA* was reported recently (García-Álvarez et al., 2011) and few studies are available on the prevalence of *mecC* in humans and animals (Diaz et al., 2016). Strains carrying the *mecC* gene have been isolated from cattle in different European countries, but not on the American continent; however, it is advisable to check for its presence to monitor its dissemination over time and to discover potential animal reservoirs (García-Álvarez et al., 2011; Petersen et al., 2013; Ariza-Miguel et al., 2014; Diaz et al., 2016).

Genetic typing revealed that the isolates presented with the same SCC*mec* type IV and belonged to sequence type 8 and *spa* type t024, related to the USA300 type, the predominant cause of CA-MRSA infection in the United States (Tenover and Goering, 2009), which is disseminated in Latin America, particularly in Colombia (Reyes et al., 2009; Márquez-Ortiz et al., 2014). The isolates we studied failed to amplify the *arcA* gene, an ACME-specific gene often found in USA300 isolates (Planet et al., 2013), but that seems to be absent in the Latin American strains (Márquez-Ortiz et al., 2014; Hidalgo et al., 2015). Moreover, USA300 isolates are showing increasing tetracycline resistance (Tenover and Goering, 2009), which can be related to the presence of ACME (Planet et al., 2013), thus explaining the lack of tetracycline resistance found in our isolates and in

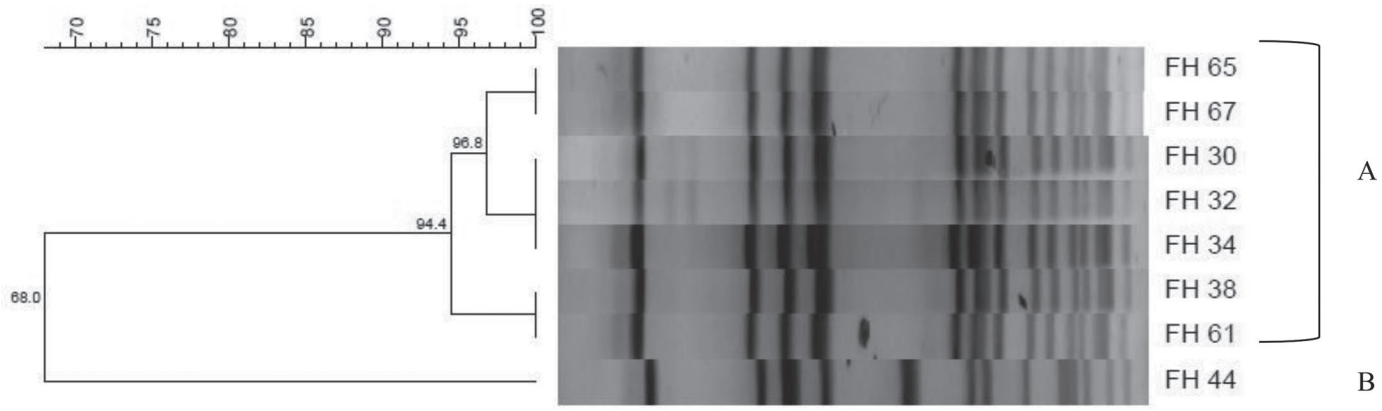

Figure 1. Dendrogram showing the similarities between pulsed-field gel electrophoresis profiles of methicillin-resistant *Staphylococcus aureus* isolates using the Dice coefficient and unweighted-pair group method using arithmetic mean.

other studies carried out with USA300-related strains isolated in Colombia (Hidalgo et al., 2015).

Seven isolates (FH 65-FH 67, FH 30-FH 32-FH 34, and FH 38-FH 61) presented 3 closely related PFGE profiles with 94.4% similarity, whereas isolate FH 44 presented a different band pattern (Figure 1). Visual comparison of pulsotypes showed similarities with those obtained by Márquez-Ortiz et al. (2014) among Colombian pediatric patients.

Three isolates (FH 61, FH 65, and FH 67) carried the SEB gene. The presence of genes responsible for SE production in food isolates of MRSA has been described by other authors (Normanno et al., 2007; Can and Çelik, 2012) and there is a reported outbreak of foodborne intoxication due to an enterotoxigenic strain of MRSA producing SEC (Jones et al., 2002). Methicillin-resistant *S. aureus* is a pathogen of concern due to the severity of the illnesses caused and its worldwide spread. Its presence in foods of animal origin adds additional threats, as it may cause staphylococcal intoxication if the strains are able to produce enterotoxin, and may contribute to the dissemination along the food chain (Doyle et al., 2011). On the other hand, the SEB is a toxin type rarely associated with staphylococcal food poisoning (Seo and Bohach, 2013). The characteristics of the isolates are summarized in Table 1.

The results of the present study show the presence of USA300-related strains of CA-MRSA in Doble Crema cheese, indicating a human source of contamination. Transmission of MRSA strains between farm workers and farm animals has been reported (Juhász-Kaszanyitzky et al., 2007; Lim et al., 2013) and also between food handlers and foods (Jones et al., 2002). The risk of human infection by MRSA via contaminated foods is considered low (EFSA, 2009), but food-related outbreaks of MRSA infection and intoxication have been reported (Kluytmans et al., 1995; Jones et al., 2002) and contaminated food commodities can contribute to the worldwide dissemination of clones of CA-MRSA (Ogata et al., 2012; Rodríguez-Lázaro et al., 2015). Improved hygienic measures in food processing plants are needed to ensure the microbiological safety of foods. More studies must be conducted to identify the sources of contamination and to implement control mechanisms.

Table 1. Genetic characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates[1]

Isolate	*mecA*	PVL	Enterotoxin gene	SCC*mec* type	PFGE cluster	MLST type	*spa* type
FH 30	+	+	–	IV	A	8	t024
FH 32	+	+	–	IV	A	8	t024
FH 34	+	+	–	IV	A	8	t024
FH 38	+	+	–	IV	A	8	t024
FH 44	+	+	–	IV	B	8	t024
FH 61	+	+	SEB	IV	A	8	t024
FH 63	+	+	SEB	IV	A	8	t024
FH 65	+	+	SEB	IV	A	8	t024
FH 67	+	+	SEB	IV	A	8	t024

[1]PVL = Panton-Valentine leukocidin; PFGE = pulsed-field gel electrophoresis; MLST = multilocus sequence typing.

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