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First isolation of a methicillin-resistant *Staphylococcus aureus* from bovine mastitis in Argentina

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ABSTRACT

This research communication describes the first isolation of a methicillin-resistant *Staphylococcus aureus* (MRSA) from cow's mastitic milk in Argentina. Bovine mastitis causes important economic losses in the dairy industry and the most commonly isolated bacteria from bovine mastitis are *staphylococci*. The *mec*A gene present in MRSA bacteria confers resistance to almost all β-lactam antibiotics, the most frequent drugs used in bovine mastitis therapy.

1. Introduction

Mastitis, the most common disease in dairy cattle and the most costly to the dairy industry, is an inflammatory reaction of the mammary gland tissue (Bradley, Leach, Breen, Green, & Green, 2007). The inflammation of the udder usually occurs in response to bacteria invading the mammary gland through teat canal. Cows with clinical mastitis present abnormalities both in the udder and in the milk, whereas those with subclinical mastitis have no visible signs of infection, and can be detected only by somatic cell count with California mastitis test. Mastitis treatment is sometimes possible with long administration of antibiotics, and milk from that treated cows is not marketable until antibiotic residues have left the cow's udder. Antibiotics may be either administered systemically or forced upwards into the mammary gland through the teat canal, the latter of which is referred to as intramammary infusion therapy. The most common causative agents isolated from milk samples collected from cows with clinical and subclinical mastitis in several countries are staphylococci. *Staphylococcus aureus* is the main pathogen among the genus, and, in some geographical areas, the one responsible for up to 40% of all mastitis cases (Barkema, Schukken & Zadoks, 2006; Bradley et al., 2007; Gentilini et al., 2000). Identification of the mastitis pathogens is important when selecting appropriate antimicrobial therapy. β-lactam antibiotics are frequently used in intramammary infusion therapy (Saran & Chaffer, 2000). However, methicillin-resistant *S. aureus* (MRSA) is resistant to all β-lactam antibiotics, excluding anti-MRSA cephalosporins, ceftobiprole and ceftaroline, because the activity of antibiotic-inhibited penicillin-binding proteins is replaced by the function of an acquired penicillin-binding protein with low affinity (García-Álvarez et al., 2011). This low affinity protein is encoded by the *mec*A or *mec*C (a *mec*A homolog) gene located on a mobile genetic element called staphylococcal chromosomal cassette (SCC*mec*) (García-Álvarez et al., 2011). SCC*mec* elements are highly diverse in their structural organization and genetic content, and have been classified into types and subtypes. Until now, 12 different types of SCC*mec* harboring *mec*A/C (types I-XII) as well as numerous subtypes have been described in staphylococci (Wu, Li, Liu, Xue & Zhaoa, 2015).

2. Case report

During June 2017 to September 2018, 150 milk samples collected from cows with clinical and subclinical mastitis from different farms in the Province of Buenos Aires (Argentina) were analyzed to determine staphylococcal resistance towards β-lactam, macrolide and lincosamide antibiotics. From these milk samples, a total of 180 staphylococci were isolated, 150 of them were identified as coagulase-negative

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Table 1
Antimicrobial susceptibility and resistance genes to β-lactam antibiotics of $n = 180$ staphylococci isolated from bovine mastitis.

Antimicrobials	CNS ($n = 150$)	*S. aureus* ($n = 30$)
PEN	26.7% ($n = 40$)	16.7% ($n = 5$)
FOX/OXA	6.7% ($n = 10$)	3.3% ($n = 1$)
Genes		
*mec*A	10	1
*mec*C	0	0

CNS: coagulase-negative staphylococci.

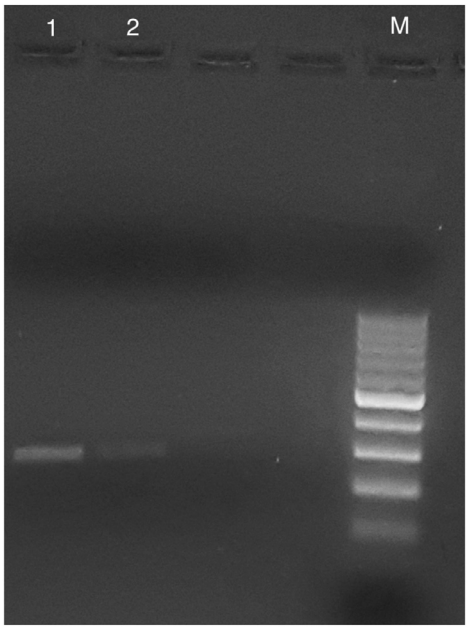

Fig 1. PCR of cassette SCC*mec* subtype IVa. 1: *S. aureus* strain P28, 2: positive control 278 bp, M: ladder marker 2 kb.

staphylococci (CNS) and 30 as *S. aureus* coagulase-positive staphylococci. Staphylococci were isolated on blood agar plates and identification of *S. aureus* was based on Gram stain, hemolysis production and biochemical reactions to catalase, coagulase, mannitol, maltose, trehalose, Voges-Proskauer and colony color in chromogenic medium (CHROMAgar). CNS isolates were identified based on additional biochemical reactions as oxidase, susceptibility to novobiocin, ONPG and fermentation of several sugars. All staphylococcal isolates were tested by disk diffusion for susceptibility to penicillin (PEN, 10 U), oxacillin (OXA, 1 μg) and cefoxitin (FOX, 30 μg), according to the guidelines of the Clinical Laboratory Standard Institute (CLSI, 2013). Cefoxitin-resistant staphylococci were tested for the *mec*A and *mec*C genes by PCR, which was carried out with the primers and conditions previously described by Zhang, McClure, Elsayed, Louie, and Conly (2005) and Cuny, Layer, Strommenger, and Witte (2011). The MBD33 strain (Cátedra de Microbiología, FCV, UBA) and the *S. aureus* LGA251 strain (García-Álvarez et al., 2011), were used as positive controls for *mec*A and *mec*C genes respectively. The antimicrobial diffusion test showed that 16.7% ($n = 5$) *S. aureus* were resistant to PEN and that 3.3% ($n = 1$) were resistant to OXA and FOX. Among the 150 CNS, 26.7% ($n = 40$) were resistant to PEN and 6.7% ($n = 10$) to OXA and FOX (Table 1). The *S. aureus* isolate that was resistant to PEN, OXA and FOX, named P28, was further identified as *Staphylococcus aureus* by *gap* gene sequencing. This strain was found to be *mec*A positive. SCC*mec* typing (Milheiriço, Oliveira & de Lencastre, 2007) revealed the presence of a cassette type IV subtype a (Fig. 1). The absence of Panton-Valentine leucocidin coding genes (*luk*S/F-PV) was determined by PCR (Lina et al., 1999).

3. Discussion and conclusions

This research work reports the first *S. aureus* isolate recovered from bovine mastitis in Argentina, positive for the *mec*A gene and resistant to β-lactam antibiotics. This finding is in accordance with many other studies in this field in other countries as Italy (Basanisi, La Bella, Nobili, Franconieri & La Salandra, 2017), Finland (Gidonis et al., 2013), Japan (Baba et al., 2012), Iran (Havaei et al., 2015), Colombia (Herrera, García-López & Santos, 2016), where they found the SCC*mec* type IV and also the absence of Panton-Valentine leucocidin in the MRSA strains. About β-lactam resistance in CNS isolates, this is low and coincides with other studies (Srednik et al., 2017).

The detection of the MRSA described here represents a significant finding because of the potential public health threat regarding antimicrobial resistance and development of multiple resistances. Some staphylococcal species in dairy cattle are also commonly found in humans (Sampimon, Lam, Mevius, Schukken & Zadoks, 2011). Thus, humans and dairy cattle may exchange bacteria, and this could provide new sources of antimicrobial resistance in human health and veterinary medicine.

The emergence and evolution of resistance is a complex and multifactorial process, which depends, among others, on the selective pressure of antibiotics from different origins. The resistance to antibiotics is a health problem of global relevance in both medicine and veterinary medicine and thus highlights the need for prudent and responsible use of antibiotics. The misuse and abuse of antimicrobials is responsible for the emergence and rapid spread of staphylococcal isolates resistant to methicillin and to other families of antibiotics (multiresistance).

Nowadays, SCC*mec* typing represents a useful tool for the study of MRSA molecular epidemiology. The different SCC*mec* types present several differences in terms of antimicrobial susceptibility and toxin distribution. SCC*mec* IV, V and VII usually carry a smaller cassette that confers resistance only to β-lactam antibiotics, whereas SCC*mec* I, II or III are usually resistant to multiple drugs (Vandenesch et al., 2003).

We strongly suggest isolating and identifying the microorganisms recovered from milk samples, determining antibiotic susceptibility and performing *mec*A/*mec*C PCR detection in the case of staphylococcal isolates resistant to oxacillin and/or cefoxitin. The benefit of this methodology would allow taking actions for the control of resistance spread such as removing positive carriers from the production and using specific antibiotics.

To our knowledge, this is the first report describing the isolation of a MRSA isolate from bovine mastitis in Argentina.

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