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 mecA 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, cefoxitin and ceftaroline, because the activity of anti-MRSA 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 mecA or mecC (a mecA homolog) gene located on a mobile genetic element called staphylococcal chromosomal cassette (SCCmec) (García-Álvarez et al., 2011). SCCmec 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 SCCmec harboring mecA/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...
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          |              |                   |
| meca           | 10           | 1                 |
| mecC           | 0            | 0                 |

CNS: coagulase-negative staphylococci.

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

3. Discussion and conclusions

This research work reports the first S. aureus isolate recovered from bovine mastitis in Argentina, positive for the meca 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 Saldarena, 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 SCCmec 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, SCCmec typing represents a useful tool for the study of MRSA molecular epidemiology. The different SCCmec types present several differences in terms of antimicrobial susceptibility and toxin distribution. SCCmec IV, V and VII usually carry a smaller cassette that confers resistance only to β-lactam antibiotics, whereas SCCmec 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 meca/mecC 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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References

Baba, K., Ishihara, K., Ozawa, M., Masaru, U., Hiki, M., Tamura, Y., et al. (2012). Prevalence and mechanism of antimicrobial resistance in Staphylococcus aureus isolates from diseased cattle, swine and chickens in Japan. The Journal of Veterinary Medical Science, 74(S), 561–565.
Basanisi, M. G., La Bella, G., Nobili, G., Franconieri, I., & La Saldarena, G. (2017). Genotyping of methicillin-resistant Staphylococcus aureus (MRSA) isolated from milk and dairy products in South Italy. Food Microbiology, 62, 141–146.
Barkema, H. W., Schukken, Y. H., & Zadoks, R. N. (2006). Invited review: The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine Staphylococcus aureus mastitis. Journal of Dairy Science, 89, 1877–1895.
Bradley, A. J., Leach, K. A., Breen, J. E., Green, L. E., & Green, M. J. (2007). Survey of the
incidence and etiology of mastitis on dairy farms in England and Wales. Veterinary Record, 160, 253–257.

Clinical and Laboratory Standards Institute. (2013). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: Approved standard and supplement VET01-A4 and VET01-S2 (Fourth Edition). Wayne, PA: Clinical Laboratory Standard Institute.

Cuny, C., Layer, F., Strommenger, B., & Witte, W. (2011). Rare occurrence of methicillin-resistant Staphylococcus aureus CC130 with a novel mecA homologue in humans in Germany. PLoS One, 6, E24360 1371/journal.pone.002436.

García-Álvarez, L., Holden, M. T., Lindsay, H., Webb, C. R., Brown, D. F. J., & Curran, M. D. (2011). Methicillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: A descriptive study. The Lancet Infectious Diseases, 11, 595–603.

Gentilini, E., Denamiel, G., Llorente, P., Godaly, S., Rebuelto, M., & DeGregorio, O. (2000). Antimicrobial susceptibility of Staphylococcus aureus isolated from bovine mastitis in Argentina. Journal of Dairy Science, 83, 1224–1227.

Gidonis, V., Taponen, S., Myllyniemi, A., Pyörälä, S., Nykäsenoja, S., Salmenlinna, S., et al. (2013). Occurrence and characterization of methicillin resistant staphylococci from bovine milk samples in Finland. Acta Veterinaria Scandinavica, 55, 61, Havaei, S. A., Assadbeigi, B., Esfahani, B. N., Hoseini, N. S., Rezaei, N., & Havaei, S. R. (2015). Detection of mecA and enterotoxin genes in Staphylococcus aureus isolates associated with bovine mastitis and characterization of Staphylococcal cassette chromosome mec (SCCmec) in MRSA strains. Iranian Journal of Microbiology, 7(3), 161–167.

Herrera, F. C., García-López, M. L., & Santos, J. A. (2016). Short communication: Characterization of methicillin-resistant Staphylococcus aureus isolated from raw milk fresh cheese in Colombia. Journal of Dairy Science, 99(10), 7872–7876.

Lina, G., Quingia, A., Reverdy, M. E., Leclercq, R., Vandenbossche, F., & Etienne, J. (1999). Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. Antimicrobial of Agents Chemotherapy, 43, 1062–1066.

Milheiro, C., Oliveira, D. C., & de Lencastre, H. (2007). Multiplex PCR strategy for subtyping the staphylococcal cassette chromosome mec type IV in methicillin-resistant Staphylococcus aureus: ‘SCCmec IV multiplex’. Journal of Antimicrobial Chemotherapy, 60, 42–48.

Sampimon, O. C., Lam, T. J. G. M., Mvevus, D. J., Schukken, Y. H., & Zadoks, R. N. (2011). Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine milk samples. Veterinary Microbiology, 150, 173–179.

Saran, A., & Chaffer, M. (2000). Tratamiento de la mastitis En. Mastitis y calidad de leche. Ed. Buenos Aires, Argentina: Intermedica73–86.

Srednik, M., Tremblay, V., Labrie, J., Archambault, M., Jacques, M., Fernandez Cirelli, A., et al. (2017). Biofilm formation and antimicrobial resistance genes of coagulase-negative staphylococci isolated from cows with mastitis in Argentina. FEMS Microbiology Letters, 364(8), https://doi.org/10.1093/femsle/fnx001.

Vandenesch, F., Naimi, T., Enright, M. C., Lina, G., Nimo, G. R., Heffernan, H., et al. (2003). Community acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: Worldwide emergence. Emerging Infectious Diseases, 9, 978–984.

Wu, Z., Li, F., Liu, D., Xue, H., & Zhaoa, X. (2015). Novel type XII Staphylococcal cassette chromosome mec harboring a new cassette chromosome recombinase, CcrC2. Antimicrobial of Agents Chemotherapy, 59, 7597–7601.

Zhang, K., McClure, J., Elsayed, S., Loebe, T., & Conly, J. (2005). Novel multiplex PCR assay for characterization and concomitant subtyping of Staphylococcal cassette chromosome mec types I to V methicillin-resistant Staphylococcus aureus. Journal of Clinical Microbiology, 43, 5026–5033.