Methicillin-Resistant *Staphylococcus aureus* Harboring mecC in Livestock in Spain

Jaime Ariza-Miguel,a Marta Hernández,a Isabel Fernández-Natal,b,c David Rodríguez-Lázaroa-d

Instituto Tecnológico Agrario de Castilla y León, Valladolid, Spain; Complejo Asistencial Universitario de León, León, Spain; Institut of Biomedicine (IBIOMED), University of León, León, Spain; University of Burgos, Burgos, Spain

We report for the first time mecC-positive methicillin-resistant *Staphylococcus aureus* (mecC-MRSA) in livestock in Spain. One isolate (sequence type 130) was found in milk samples among 601 *S. aureus* isolates obtained from 229 dairy sheep farms. This finding highlights the potential for zoonotic transmission of mecC-positive MRSA and the need for surveillance programs to monitor its presence and clonal evolution.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of nosocomial infections ranging from mild skin and soft tissue infections to life-threatening diseases, such as sepsis, toxic shock syndrome, endocarditis, and necrotizing pneumonia. Resistance to methicillin is mediated by the presence of the mecA gene, which encodes an additional penicillin-binding protein, PBP2a, with only a poor affinity for β-lactam antibiotics. A homologous gene, mecC (formerly mecA_{LAI251}), was recently described (1, 2). Because of its divergence from mecA (only 69% and 63% identity at the DNA and amino acid levels, respectively), many standard and commercial diagnostic methods fail to detect mecC-positive MRSA (mecC-MRSA), leading to inappropriate treatment and underestimations of the prevalence of MRSA in clinical and environmental settings.

Since then, mecC-MRSA isolates have been detected in 13 European countries (Austria, Belgium, Denmark, Finland, France, Germany, Norway, the Republic of Ireland, Spain, Sweden, Switzerland, The Netherlands, and the United Kingdom) and have been isolated from 14 animal species, including companion, livestock, and wildlife animals (3). Evidence has been presented for the zoonotic potential of mecC-MRSA lineages (4, 5). It is therefore important to monitor potential animal reservoirs for the presence of such lineages.

The presence of mecC-MRSA was recently reported for the first time in clinical and environmental settings in Spain (6–8), but the prevalence of mecC-MRSA in livestock and its potential routes of human transmission are unknown. Spain has the second highest number of sheep among the 28 European Union members and 20% of the total European sheep population (http://www.coag.org). Here, we report our evaluation of the presence of mecC-MRSA in Spanish dairy sheep farms.

We performed a retrospective study for mecC- and mecA-positive MRSA among a collection of 601 *S. aureus* isolates obtained from bulk milk samples collected from 229 dairy sheep farms from August 2008 to July 2009. Up to three isolates per farm were analyzed. The farms sampled were in 10 provinces (Ávila, Burgos, Cáceres, León, Madrid, Palencia, Salamanca, Segovia, Valladolid, and Zamora) that are within the Spanish geographical area with the highest sheep milk production (60% of the nation’s sheep milk production). Screening for the presence of mecA and mecC was performed by multiplex PCR (9) on confirmed *S. aureus*-positive colonies in Baird Parker medium. A sample from 1 (0.44%) of 229 farms (1 of 601 *S. aureus* isolates) contained mecC-MRSA, and 3 (1.31%) farms (9 of 601 isolates) tested positive for mecA-MRSA. The characteristics of the MRSA isolates harboring mecC and/or mecA are detailed in Table 1. The rarity of mecC-MRSA observed in this study agrees with the findings of previous studies in European livestock (10). mecC has also been found occasionally in human MRSA infections in Europe; one report found mecC-MRSA in 0.06% to 2.8% of the isolates tested (3). A retrospective study in Spain recently identified five mecC-MRSA isolates among isolates collected from one hospital between 2008 and 2013. In the same work, retrospective screening for mecC among 5,505 human *S. aureus* isolates received during the same period at the Spanish National Reference Centre for *Staphylococci* revealed two additional mecC-MRSA isolates (0.036%) (6).

The antimicrobial susceptibility of each mecA- and mecC-positive MRSA isolate was tested for 22 antimicrobial agents by a microdilution method following the recommendations and MIC breakpoints of the 2013 EUCAST guidelines v3.1 (see http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_3.1.pdf) (Table 1). The mecC-positive isolate was sensitive to all non-β-lactam antibiotics tested, as reported for other similar mecC-carrying isolates (3, 10). An interesting finding was that all mecA-MRSA isolates were resistant not only to β-lactams but also to tetracycline (Table 1). The mecC isolates belonging to clonal complex 130 (CC130) have not generally acquired further resistance determinants, although resistance to ciprofloxacin has been reported sporadically (11). This suggests that the lineage has not been extensively subjected to antibiotic selective pressure, consistent with its low prevalence in the clinical environment. Further studies to clarify this issue are required.

The genetic backgrounds of all the MRSA isolates were investigated by multilocus sequence typing (MLST) (6). The genetic struc-
ture of the staphylococcal cassette chromosome mec element (SCCmec) and the presence of Panton-Valentine leukocidin (PVL) virulence factor genes ( lukS-PV and lukF-PV) were also determined (2, 12-14). MLST analysis revealed that the mecC-carrying isolate belongs to sequence type 130 (ST130) (CC130). Most mecC-MRSA isolates analyzed belong to CC130, irrespective of the geographic origin or the host from which they were recovered. ST425 has also been reported, but less frequently, and other genetic backgrounds have been described sporadically (3). Characterization of SCCmec revealed that the mecC isolate harbored SCCmec type XI, as previously reported for all mecC-MRSA isolates studied to date. PVL virulence genes were not detected, which was not surprising, as all tested mecC-MRSA strains have been negative for this virulence factor (3). These various observations suggest that mecC-MRSA is still rare in clinical and environmental settings and that this lineage has only limited genetic diversity. This is an interesting conclusion because although the lineage was first described in 2011, mecC-MRSA has been circulating freely for at least the last 35 years (1). MLST analysis indicated that the mecA-MRSA isolates belong to ST398, which is strongly associated with livestock, as it was recovered from pigs in several European countries. The mecA-MRSA isolates from farms 741 and 1043 harbored SCCmec type V, and isolates from farm 1040 were of subtype IVa (Table 1). PVL virulence genes were not detected.

The zoonotic potential of mecC-MRSA has been formally demonstrated (4, 5). However, there is little information available about the role of livestock in the transmission of mecC-MRSA to humans (4), and there has been no evidence, until now, of the presence of this lineage in livestock in Spain. The two largest sheep populations in Europe are in the United Kingdom and Spain (see http://www.coag.org). A recent study found that 2.15% of dairy cattle farms in England and Wales were positive for mecC, but interestingly, no mecC-positive farms were found in Scotland (14). However, there are no studies about the prevalence of mecC MRSA on sheep farms in Europe. Our study is the first to detect mecC-MRSA in livestock (dairy sheep farms) in Spain. This study was retrospective, and the sampling was not performed following a formal randomization process, so the results cannot be directly interpreted for prevalence values; however, this information provides an overview and baseline values that can be used in prospective prevalence studies.

In conclusion, we report for the first time the presence of mecC-MRSA in livestock in Spain. Although this lineage seems to be rare (0.44% of farms tested), it is of particular public health relevance because of its zoonotic potential, its ability to cause life-threatening disease (4, 6), and our inability to detect it with standard testing procedures. This demonstration that mecC-MRSA is present in livestock in Europe, and now in Spain in particular, highlights the need for surveillance programs; the presence and evolution of mecC-MRSA in animal and environmental reservoirs need to be monitored.

ACKNOWLEDGMENTS

This work was supported by the European Union 7th Framework Program under the project PROMISE (project number 265877). We thank Bruno Gonzalez-Zorn (Universidad Complutense de Madrid) for kindly providing mecC-MRSA-positive control samples.

REFERENCES


