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

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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 septicemia, 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 $mecA_{LGA251}$), 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 (see 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-

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TABLE 1 Characteristics of *mecC*- and/or *mecA*-positive MRSA isolates from dairy sheep farms, Spain, 2008–2009

Resistance determinant	Farm code (no. of isolates[a])	Date of isolation (mo/day/yr)	Region	MLST	SCC*mec*	MRSA clone	Antibiotic resistance[b]
mecC	648 (1)	3/03/2009	Zamora	ST130	XI	ST130-MRSA-XI	β-Lactams only
mecA	741 (3)	4/07/2009	Palencia	ST398	V	ST398-MRSA-V	β-Lactams, Tet
mecA	1040 (3)	7/28/2009	Palencia	ST398	IVa	ST398-MRSA-IV	β-Lactams, Tet
mecA	1043 (3)	7/28/2009	Valladolid	ST398	V	ST398-MRSA-V	β-Lactams, Tet

[a] Three isolates from each farm were analyzed.
[b] The antibiotic panel included penicillin, oxacillin, amoxicillin-clavulanate, daptomycin, cefazolin, erythromycin, clindamycin, teicoplanin, vancomycin, ciprofloxacin, levofloxacin, amikacin, gentamicin, tobramycin, mupirocin, rifampin, tetracycline (Tet), fusidic acid, fosfomycin, nitrofurantoin, linezolid and co-trimoxazole.

ture of the staphylococcal cassette chromosome *mec* element (SCC*mec*) 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 SCC*mec* revealed that the *mecC* isolate harbored SCC*mec* 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 SCC*mec* 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.

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