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Staphylococcus aureus Carrying *mec*C Gene in Animals and Urban Wastewater, Spain

To the Editor: A new methicillin resistance mechanism gene, a divergent *mec*A homologue named *mec*C (formerly *mec*A_{LGA251}), was recently described in *Staphylococcus aureus* (*1*). Methicillin-resistant *S. aureus* (MRSA) isolates carrying *mec*C have been recovered from humans, ruminants, pets, and other animals such as rats, seals, and guinea pigs (*1–3*). It has been suggested that *mec*C-carrying MRSA isolates might not be detected by using MRSA selective media (*4*). For *mec*C-carrying *S. aureus* isolates, cefoxitin MICs of 4–64 mg/L have been demonstrated (*1–2*,*4*), values that would normally include susceptible isolates, according to the epidemiologic cutoff value established by the European Committee on Antibiotic Susceptibility Testing (EUCAST; www.eucast.org). *mec*C-carrying *S. aureus* isolates have been classified as heteroresistant (*5*), and MICs can be affected by the drug-susceptibility testing method used (*1,5*).

These observations led us to retrospectively investigate the presence of *mec*C gene in a set of 361 *mec*A-negative *S. aureus* isolates collected during 2009–2012 (Table), independently of their susceptibility to cefoxitin. Isolates were recovered from healthy carriers in livestock (n = 39), from wild animals (n = 254), and from wastewater (effluents) from an urban sewage plant (n = 68). Specific amplification of the *mec*C gene was performed as described (*6*). The *mec*C-carrying *S. aureus* isolates were tested by broth microdilution using Microtiter EUST plates (Trek Diagnostic Systems, East Grinstead, UK) for susceptibility to benzylpenicillin, cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, florfenicol, fusidic acid, gentamicin, kanamycin, linezolid, mupirocin, rifampin, sulfamethoxazole, streptomycin, quinupristin-dalfopristin, tetracycline, thiamulin, trimethoprim, and vancomycin. Additionally, susceptibility to oxacillin was determined by using microScan Gram Positive Combo panel 37 (Siemens, Erlangen, Germany). MICs were interpreted according to EUCAST epidemiologic cutoff values.

*mec*C was detected in a total of 4 isolates from wild boar (n = 1), fallow deer (n = 2), and urban wastewater (n = 1); these isolates represent 1% of the 361 tested isolates. The 3 isolates recovered from animals were susceptible to all antimicrobial drugs tested other than β-lactams and to oxacillin (MICs 0.5–1 mg/L) but were resistant to penicillin (MICs 0.5–2 mg/L). Two of the isolates were resistant to cefoxitin (MICs 8 and 16 mg/L) and the third was susceptible (MIC 4 mg/L). The wastewater isolate was resistant to penicillin (MIC 2 mg/L) and erythromycin (MIC 16 mg/L) and susceptible to all other antimicrobial drugs tested, including cefoxitin (MIC 4 mg/L) and oxacillin (MIC ≤0.25 mg/L).

Table. Testing of *Staphylococcus aureus* isolates for presence of methicillin resistance mechanism gene *mec*C, Spain*

Isolate source	Year(s) of isolation	No. *mec*C-positive isolates	*spa* type	MLST	CC	Antimicrobial resistance profile
Livestock, n = 39						
Cattle, n = 5	2011	0				
Fattening pigs, n = 34	2009, 2011	0				
Wild animals, n = 254						
Eurasian griffon vulture, n = 2	2011	0				
Fallow deer, n = 2	2012	2	t11212	ST425	CC425	PEN, FOX
			t11212	ST425	CC425	PEN
Iberian ibex, n = 39	2009–2010	0				
Mouflon, n = 2	2009	0				
Red deer, n = 61	2009–2011	0				
Wild boar, n = 148	2009–2011	1	t11212	ST425	CC425	PEN, FOX
Urban wastewater, n = 68	2011	1	t843	ST2676	CC130	PEN, ERY

*MLST, multilocus sequence typing; ST, sequence type; CC, clonal complex; PEN, benzylpenicillin; FOX, cefoxitin; ERY, erythromycin.

Previous studies have described *mec*C-positive isolates as susceptible to all antimicrobial drugs tested except β-lactams (*2,3*), although sporadic resistance to fluoroquinolones has been found (*4,7*). We additionally found erythromycin resistance in 1 *mec*C-carrying *S. aureus* isolate. For the 4 *mec*C-carrying *S. aureus* isolates we detected, MICs of oxacillin were interpreted as susceptible, and 2 isolates were susceptible to cefoxitin according to EUCAST guidelines, findings that agree with previous reports (*1–2,4*). Thus, *mec*C presence is not always linked to resistance phenotypes for cefoxitin or oxacillin; such unclear findings could hinder the detection of *mec*C-carrying isolates.

We further characterized the 4 *mec*C-carrying *S. aureus* isolates by *spa* typing and detection of Panton-Valentin leukocidin (PVL) toxin genes (*6,8*). Multilocus sequence typing (MLST) was performed according to Enright et al. (*9*) by using self-designed primers *arc* (down 5′-CGATTTGTTGTTGATTAGGTTC-3′), *tpi* (up 5′-CATTAGCAGATTTAGGCGTTA-3′), and *yqi*L (down 5′-GATTGGYTCACCTTTRCGTTG-3′). All 4 isolates were PVL negative. The 3 animal isolates were assigned to a new *spa* type (t11212) and to clonal complex (CC) 425 and sequence type (ST) 425 (Table). ST425 has been previously associated with *mec*C-carrying *S. aureus* isolates in cattle and humans (*1–2*); the animals we sampled were from a game estate and may have had contact with cattle and with urban wastewater. The wastewater isolate was assigned to *spa* type t843 and to a new allelic profile, ST2676, in CC130 (Table). ST2676 represents a single-locus variant of ST130 carrying a different allele for the gene *aro*E. MRSA isolates of CC130 have been associated with humans and animals (*1–4,6*). This result indicates that *mec*C-carrying *S. aureus* isolates can be found in urban wastewater, which may act as an environmental reservoir, as has been demonstrated for *mec*A-carrying *S. aureus* (*10*).

In conclusion, we detected the methicillin resistance mechanism gene *mec*C in nonclinical *S. aureus* isolates from animals and urban wastewater in Spain. Although our data indicate that the frequency of this resistance mechanism is low, this gene appears to be expanding to new areas. Prospective studies should be performed to evaluate epidemiologic changes and to analyze the genetic lineages that carry this resistance mechanism.

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Schmallenberg Virus Antibodies in Adult Cows and Maternal Antibodies in Calves

To the Editor: Schmallenberg virus (SBV), a novel orthobunyavirus that is transmitted by *Culicoides* spp. biting midges, spread through herds of ruminants across Europe during 2011–2013. The virus reached as far as Finland in the north, the Republic of Ireland in the west, Turkey in the east (*1*), and Spain in the south. The clinical effect of SBV infection in ruminant livestock appears to be limited (*2*), and a vaccine to prevent the infection has been developed (*3*). There are no data to refute the assumption that natural SBV infection results in long-term immunity, as was seen earlier with natural infection of cattle with bluetongue virus serotype 8 (*4*). Newborn calves acquire passive immunity by ingestion and absorption of antibodies present in colostrum. Passive immunity can, however, block the production of serum antibodies when vaccine is administered to calves that have maternally derived antibodies (*5*). To determine the titers and persistence of SBV antibodies in adult cows and the decay of maternal antibodies in calves over time, we studied a herd of cattle from a dairy farm in the eastern Netherlands during April 2012–April 2013.

The dairy farm is the only location in the Netherlands where monitoring for biting midges was continuously conducted during the 2011–2013 SBV epidemic and where SBV RNA was detected in biting midges caught during 2011–2012 (*6,7*). The dairy herd comprised 110 animals: 60 milking cows (average age 4.0 years) and 50 heifers (average age 1.5 years) and calves (<1.0 year of age). No clinical signs or symptoms of SBV infection were observed in any of the cattle at the end of 2011 or during 2012. However, during the study period, 3 calves were stillborn, none of which had the characteristic malformations observed after SBV infection. Gross pathology confirmed that the calves did not have SBV infection, and all tissue samples were negative for SBV by reverse transcription PCR.

During the 12-month study, we obtained 4 blood samples from all animals in the herd. A virus neutralization test (VNT) was used to test the samples for antibodies (*8*). For optimal specificity and sensitivity, the VNT cutoff dilution was set at 1:8. Test dilutions ranged from 1:4–1:512. All samples were tested in duplicate; titers were determined using the Reed-Münch method and expressed on a $\log_2$ scale.

Blood samples were first obtained from the herd on April 19, 2012, after retrospective detection of SBV RNA in biting midges that had been collected from the farm on September 14, 2011 (*6*). The remaining 3 blood samples for each animal were collected on September 17, 2012; December 9, 2012; and April 23, 2013 (5, 8, and 12 months, respectively, after the first collection). SBV VNT results for the initial blood samples were positive for all cows ≥1 year of age and for all but four 6-month-old calves. One year later, blood samples for 98% of the cows ≥1 year of age and 50% of the cows <1 year of age were SBV seropositive. During the year, the mean $\log_2$ VNT titer of the adult cows dropped from 8.3 to 6.7.

It can be assumed that cows ≥1 year of age became infected with SBV around the time SBV-infected *Culicoides* biting midges were detected on the farm in September 2011 (*6*). Thus, at least 19 months after natural infection, these cows were probably protected against SBV when re-exposed to the virus. Of all cattle tested, 11 heifers seroconverted between April 2012 and September 2012, and 1 cow seroconverted between the September and December 2012 samplings. The low rate of seroconversion was matched by a 6× lower