

Detection of methicillin-resistant *Staphylococcus aureus* ST398 in food samples of animal origin in Spain

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Keywords: MRSA, food microbiology, ST398, ST125, ST217

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Sir,
Methicillin-resistant *Staphylococcus aureus* (MRSA) strains belonging to clonal lineage sequence type (ST) 398 are being reported at an increasing frequency in Europe.¹ This new MRSA type has been isolated from colonized and infected animals and humans, and also from meat in some countries,^{1,2} representing a risk to human health; nevertheless, so far, no data about detection of MRSA ST398 in food in Spain have been published.

A total of 318 raw food samples of food-producing animals (148 chicken, 55 pork, 46 veal, 19 lamb, 10 turkey, 8 rabbit and 12 minced-meat samples) and of wild animals (8 game bird, 4 wild boar, 4 deer and 4 hare samples) were collected from November 2007 to March 2009 in La Rioja (Spain). Samples were suspended in saline solution and 100 µL was inoculated in brain heart infusion (BHI) broth containing 6.5% NaCl, and incubated at 35°C for 24 h. Then, 300 µL was seeded on ORSAB plates (Oxoid) with oxacillin (2 mg/L), and incubated at 35°C for 36 h. One blue presumptive MRSA colony per sample was selected and identified by DNase assay and by PCRs of the *mecA* and *nuc* genes.^{3,4}

MICs of 10 antibiotics were determined by the agar dilution method; the disc diffusion method was used for 7 additional antibiotics (Table 1). The presence of resistance genes was studied by PCR.⁴ Mutations in quinolone targets were determined by sequence analysis of *grrA* and *gyrA* genes.⁵ Recovered MRSA isolates were characterized by multilocus sequence typing (MLST) (saureus.mlst.net), staphylococcal cassette chromosome *mec* (SCC*mec*) typing,³ *spa* typing (www.ridom.com) and *agr* typing.⁶ The presence of Pantón–Valentine leucocidin (PVL) genes was investigated by PCR.⁶

MRSA was detected in 5 of the 318 (1.6%) food samples (from pork, chicken, rabbit, veal and wild boar). MRSA strains were characterized and the results are summarized in Table 1. The two strains from pork and veal corresponded to ST398-SCC*mecV* (*spa* types t011 and t1197, respectively), the two strains from chicken and rabbit were typed as ST125-SCC*mecIVa*-t067, and the strain from a wild boar was ST217-SCC*mecIVa*-t032. All MRSA were PVL negative.

MRSA has also been isolated from meat in other countries, and the percentages detected varied widely (0%–11%). The rate found in our study (1.6%) is in the lower range of the reported data, and higher percentages have recently been reported in the Netherlands and the USA.^{2,3} A correlation between meat type and rate of MRSA contamination cannot be established as the numbers of samples of different origins in our study differ significantly. The detection of the MRSA ST217 strain in one of the four tested wild boar samples is noteworthy. A previous study had also reported non-ST398 MRSA in food derived from game in a low percentage of samples (2.2%).²

Although MRSA prevalence in raw food is low, the risk of its transmission through the food chain cannot be disregarded, especially in uncooked meat. Indeed, foodborne disease outbreaks caused by MRSA have been reported.⁷ Moreover, contaminated foods may also constitute a health risk for food handlers. Carcasses obtained from animals colonized by MRSA may become contaminated during slaughter and foods can also become contaminated during processing by food handlers colonized by MRSA. Molecular typing is a useful tool for understanding the origin of these strains. In our study two MRSA strains were ST398, suggesting that animals could be the source of contamination. Both ST398 strains presented resistance to tetracycline, a characteristic of animal-related MRSA. Tetracycline is the most widely used antibiotic in the pig industry; macrolides and aminoglycosides are also used, but less frequently. The origin of ST398 is unclear, but the excessive use of certain antibiotics in animal production might be implicated in its emergence. A reservoir of MRSA ST398 seems to be present in food-producing animals and a high rate of nasal carriers has been found in humans in contact with these animals, which may have consequences for human health.¹ ST398 has been detected in several European countries, America and Asia. Due to the significant spread of this variant, it was expected to also be present in Spain, but, to our knowledge, this is the first report of ST398 in foods in a Mediterranean country.

The other non-ST398 strains in our study were ST125-t067 and ST217-t032, types associated with human infections, suggesting a possible human origin, and might have been transmitted by colonized food handlers. According to a recent publication, MRSA ST125-t067 was implicated in >50% of the invasive infections in Spanish hospitals.⁶ Moreover, those authors described that simultaneous resistance to ciprofloxacin, tobramycin and erythromycin is frequently found in isolates belonging to *spa*-t067, a variant carrying *ant(4′)-Ia* and *msrA* genes.⁶ One of our ST125-t067 strains presented all these characteristics. On the other hand, ST217 is a variant of epidemic EMRSA-15⁵ and was reported to present the characteristics of nosocomial MRSA with a high level of ciprofloxacin resistance, which is in accordance with our results.

In conclusion, MRSA was detected in 1.6% of meat samples in our study. Strain characterization suggests that they could be from both animal and human origin. Although the presence of MRSA in food is low, it has to be monitored because it can contribute to the spread of MRSA. To our knowledge, this is the first study concerning the prevalence of MRSA in food in Spain.

Table 1. Characteristics of the five MRSA strains^a isolated from food samples in this study

Origin	MLST/spa	SCC _{mec}	agr	MIC (mg/L)										Amino acid change		Resistance genes detected	
				OXA	FOX	TET	TOB	TOB	STR	ERY	ERY	CLI	CIP	LEV	GrlA		GyrA
Pork	ST398/t011	V	I	8	16	128	128	0.25	2	256	>256	>128	0.125	0.125	NP	NP	mecA, tet(K), tet(M), erm(A), erm(C)
Veal	ST398/t1197	V	I	64	32	128	128	32	32	8	>256	>128	2	0.5	S80F	S84L	mecA, tet(K), tet(L), tet(M), ant(4')-Ia, aph(3'), erm(C), msr(A)
Chicken	ST125/t067	IVa	II	128	128	0.25	>256	>256	128	8	0.25	0.06	64	4	S80F	S84L	mecA, ant(4')-Ia, aph(3')
Rabbit	ST125/t067	IVa	II	128	128	0.25	>256	>256	128	8	>256	>128	64	4	S80F	S84L	mecA, ant(4')-Ia, aph(3'), erm(A), erm(C), msr(A)
Wild boar	ST217/t032	IVa	I	32	32	0.125	0.25	0.25	2	8	0.25	0.06	16	4	S80F	S84L	mecA

OXA, oxacillin; FOX, cefoxitin; TET, tetracycline; TOB, tobramycin; KAN, kanamycin; STR, streptomycin; ERY, erythromycin; CLI, clindamycin; CIP, ciprofloxacin; LEV, levofloxacin; NP, not performed.

^aThe five strains were susceptible to the following seven antibiotics by the disc diffusion method: gentamicin; chloramphenicol; trimethoprim/sulfamethoxazole; vancomycin; teicoplanin; mupirocin; and fusidic acid.

Unveiling the role of MRSA ST398 as a zoonotic foodborne pathogen requires more research.

Funding

This study was financially supported by Project SAF2009-08570 from the Ministerio de Ciencia e Innovación of Spain. C. L. has a fellowship from the Ministerio de Educación y Ciencia of Spain, and M. L. and E. G.-S. have a fellowship from the Gobierno de la Rioja of Spain.

Transparency declarations

None to declare.

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Journal of Antimicrobial Chemotherapy

doi:10.1093/jac/dkp374

Advance Access publication 16 October 2009

Molecular characterization of group B streptococci with reduced penicillin susceptibility recurrently isolated from a sacral decubitus ulcer

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