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VETERINARY MICROBIOLOGY - SHORT COMMUNICATION

First report of meticillin-resistant *Staphylococcus aureus* harboring *mecC* gene in milk samples from cows with mastitis in southeastern Brazil

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Abstract

MRSA infection and colonization have been reported in both companion and food-chain animals, highlighting MRSA as an important veterinary and zoonotic pathogen. Another *mec* allele, the *mecC* gene, also confers beta-lactam resistance in *Staphylococcus aureus* and shows 69% nucleotide identity to *mecA*. The main aim of this study was to investigate the genotypic and clonal profile of methicillin-resistant *S. aureus* (MRSA) from cows with mastitis in dairy herds. Thirty-five samples suggestive of bovine subclinical mastitis were evaluated, and *S. aureus* were detected in all of them using both phenotypic and molecular approaches. According to the multilocus sequence typing (MLST), the *S. aureus* isolates were assigned in five different STs, 21 (60%) showed ST 742, 6 (17%) ST97, 4 (11%) ST1, 2 (6%) ST30, and 2 (6%) ST126. The presence of *mecA* was not observed in any of these isolates whereas *mecC* was detected in nine of them (9/35; 26%). The Panton-Valentine leukocidin (PVL) genes were detected in a total of 4 isolates. Among the 35 isolates analyzed, 26 showed resistance to penicillin. Changes in the *S. aureus* epidemiology due to the detection of MRSA in milk samples from cows presenting with bovine subclinical mastitis may have consequences for public health in Brazil, challenging the empirical therapy and animal management, with potential medical and social outcomes. To the best of our knowledge, this is the first report describing *mecC* MRSA in Southeastern Brazil.

Keywords *Staphylococcus aureus* · MRSA · mecC · Bovine mastitis

Methicillin-resistant *S. aureus* (MRSA) are still significant human and animal pathogens, causing serious and lethal infections, with inestimable public health consequences and major economic impacts [1]. MRSA strains normally contain *mecA*, a gene that encodes for the penicillin-binding protein 2a (PBP2a). This modified enzyme induces resistance to virtually all β-lactam antibiotics, making MRSA a global public health concern [2].

MRSA cases have emerged in production animals in the last years [3]. These bacteria can be transferred to man by direct contact or as food contaminants [4]. A *mecA* homolog in *S. aureus*, the *mecC*, which confers resistance to beta-

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Table 1 Primers used to amplify the *mecC* and *mecA* genes in MRSA isolates recovered from bovine mastitis

Genes	Sequence	Size	Reference
mecC (*mec*$_{LGA251}$)	f: gctcctaatgctaatgca r: taagcaataatgactacc	304pb	[12]
mecA	f: gctcctaatgctaatgca r: taagcaataatgactacc	162pb	[13]
Luk-PV1 *Luk-PV2*	atcattaggtaaaatgtctggacatgatcca gcatcaaatgtattggatagcaaaagc	463pb	[14]

lactams through a similar mechanisms (production of a PBP2a/2′ with about 63% identity at the amino acid level), was first reported in Denmark and more recently in Czech Republic [3, 5]. The *mecC* allele has been identified in the so-called livestock-associated-MRSA (LA-MRSA) belonging to different MLST clonal complexes [6, 7]. LA-MRSA has been identified in milk and beef from different livestock animals [3, 8], reinforcing MRSA as a serious threat to public health worldwide. In the present study, we investigated the genotypic and clonal profiles of methicillin-resistant *S. aureus* (MRSA) from cows presented with mastitis in a dairy herd from the states of Minas Gerais, Rio de Janeiro, and São Paulo.

A total of 35 staphylococci isolates were obtained from milk samples (15 from Minas Gerais, 15 from Rio de Janeiro, and 5 from São Paulo) from cows presented with subclinical mastitis. All milk samples were obtained from

Table 2 Characterization of *Staphylococcus aureus* isolates associated with bovine mastitis in the states of Rio de Janeiro, Minas Gerais, and Sao Paulo (Brazil)

Isolates	Region	Id.	resistance profile	MR	*Spa* type	ST	CC
MB01	MG	*S. aureus*	pen		t605	ST742	CC97
MB02	MG	*S. aureus*	cli-pen	*mecC* +	t605	ST742	CC97
MB03	MG	*S. aureus*	gen-pen-tet		t1298	ST30	CC30
MB04	RJ	*S. aureus*	pen		t605	ST742	CC97
MB05	RJ	*S. aureus*	–		t591	ST126	CC97
MB06	RJ	*S. aureus*	cli-eri-pen		t605	ST742	CC97
MB07	RJ	*S. aureus*	pen-tet		t605	ST742	CC97
MB08	SP	*S. aureus*	pen		t521	ST97	CC97
MB09	SP	*S. aureus*	pen		t605	ST742	CC97
MB10	RJ	*S. aureus*	clo-cli-eri-gen-nit-pen-rif-tet	*mecC* +	t267	ST97	CC97
MB11	MG	*S. aureus*	pen		t605	ST742	CC97
MB12	SP	*S. aureus*	pen	*mecC* +	t605	ST742	CC97
MB13	SP	*S. aureus*	pen		t1298	ST30	CC30
MB14	RJ	*S. aureus*	pen	*mecC* +	t605	ST742	CC97
MB15	MG	*S. aureus*	pen	*mecC* +	t359	ST97	CC97
MB16	MG	*S. aureus*	pen	*mecC* +	t359	ST97	CC97
MB17	MG	*S. aureus*	–		t605	ST742	CC97
MB18	MG	*S. aureus*	–		t605	ST742	CC97
MB19	MG	*S. aureus*	pen-tet		t605	ST742	CC97
MB20	RJ	*S. aureus*	nit-pen	*mecC* +	t605	ST742	CC97
MB21	RJ	*S. aureus*	pen	*mecC* +	t521	ST97	CC97
MB22	RJ	*S. aureus*	pen		t605	ST742	CC97
MB23	MG	*S. aureus*	–		t127	ST1	CC1
MB24	RJ	*S. aureus*	pen-tet		t605	ST742	CC97
MB25	MG	*S. aureus*	–		t127	ST1	CC1
MB26	MG	*S. aureus*	–		t127	ST1	CC1
MB27	SP	*S. aureus*	pen		t605	ST742	CC97
MB28	SP	*S. aureus*	oxa-pen		t605	ST742	CC97
MB29	MG	*S. aureus*	pen		t605	ST742	CC97
MB30	RJ	*S. aureus*	eri-nit-pen-sut		t605	ST742	CC97
MB31	SP	*S. aureus*	pen		t605	ST742	CC97
MB32	MG	*S. aureus*	–		t127	ST1	CC1
MB33	MG	*S. aureus*	cli-eri-pen-tet		Non-typeable	ST126	CC97
MB34	RJ	*S. aureus*	pen		t605	ST742	CC97
MB35	RJ	*S. aureus*	eri-cli-pen-tet	*mecC* +	t359	ST97	CC97

MG Minas Gerais. *SP* São Paulo, *RJ* Rio de Janeiro, *CFO* cefoxitin, *CLO* chloramphenicol, *CLI* clindamycin, *ERI* erythromycin, *GEN* gentamicin, *NIT* nitrofurantoin, *PEN* penicillin, *RIF* rifamycin, *SUT* trimethoprim-sulfamethoxazole, *TET* tetracycline, *MR* methicillin-resistance, *ST* sequence type, *CC* clonal complex

April 2015 to December 2015 and kindly provided by Brazilian Agricultural Research Corporation (EMBRAPA) Dairy Cattle, Juiz de Fora, MG. Isolates were confirmed as *S. aureus* by mass spectrophotometer in a MALDI-TOF (matrix-assisted laser desorption ionization–time off flight—Biotyper-Bruker) and PCR methodology previously described [9].

Antimicrobial susceptibility tests were performed using agar disk-diffusion method on Mueller Hinton agar (Difco), for all *S. aureus* isolates [10, 11]. The following susceptibility disks were used: cefoxitin (CFO-30 μg), chloramphenicol (CLO-30 μg), ciprofloxacin (CIP-5 μg), clindamycin (CLI-2 μg), erythromycin (ERI-15 μg), gentamicin (GEN-10 μg), rifamycin (RIF-5 μg), tetracycline (TET-30 μg), trimethoprim-sulfamethoxazole (SUT-23,75 μg), nitrofurantoin (NIT-300 μg), and penicillin (PEN-10 U). Also, the MIC for vancomycin was determined by using the Oxoid® M.I.C. Evaluator Strips ™ (M.I.C.E., Thermo Fisher Scientific, Basingstoke, UK) and broth microdiluting using microtiter plates [10].

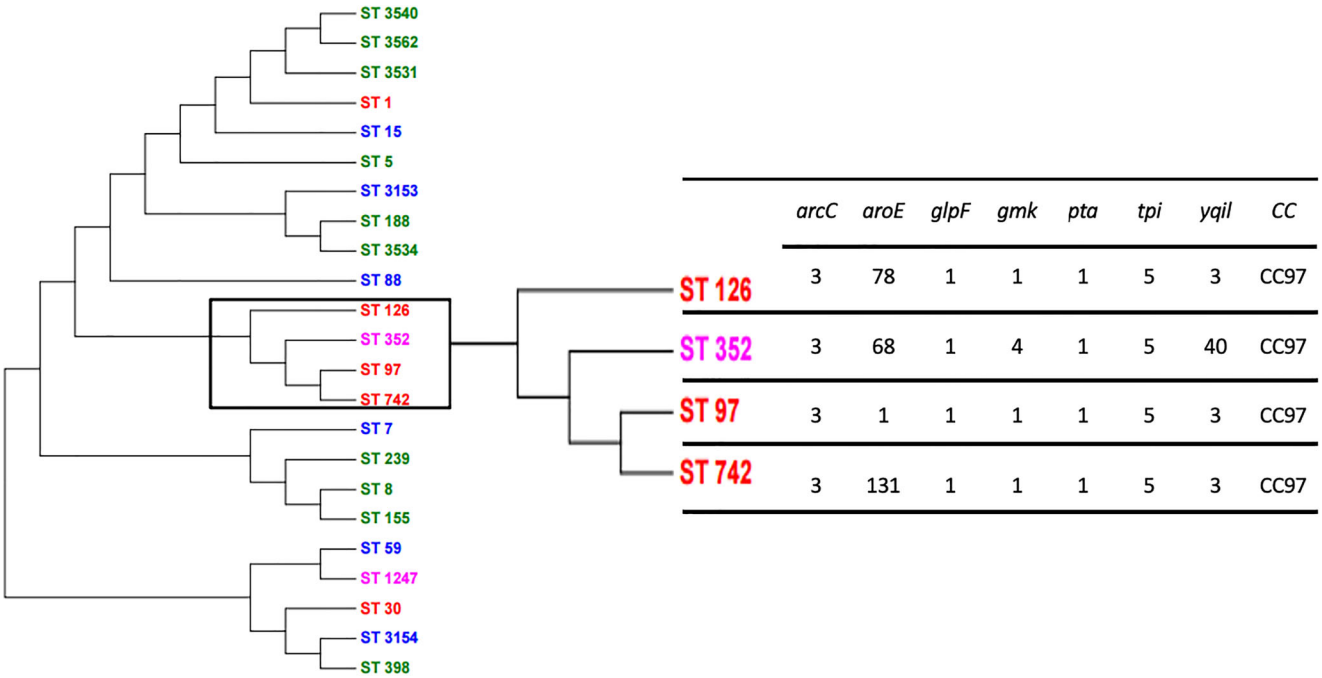
Detection of *mecA*, *mecC*, and *lukSF*-PVL genes were performed by PCR-based tests as described previously [12–14]. All primer sequences are listed in Table 1. Methicillin-resistant *S. aureus* (MRSA) strains were also characterized by performing multilocus sequence typing (MLST) [15] and spa typing [16]. To assign the MLST sequence types, the allele sequences were trimmed and analyzed using the *S. aureus* MLST database (http://www.pubmlst.org). Sequence analysis and phylogeny were performed using the BioEdit Sequence Alignment Editor v7.2.5. Phylogenetic trees were constructed by Maximum Likelihood Tree using MEGA v7.0.21.

S. aureus isolates were mostly resistant to penicillin (15 isolates). Also, eight isolates were resistant to tetracycline. In addition, all *S. aureus* detected were considered susceptible to vancomycin. Results of susceptibility are depicted in Table 2. The presence of *mecA* could not be observed in any isolate. Notably, *mecC* was detected in nine *S. aureus* isolates (26%). The Panton-Valentine leukocidin (PVL) encoding genes were detected in a total of four isolates (11%).

The total *S. aureus* isolates from mastitis were assigned in five different sequence types (STs), whereas 21 isolates (60%) were ST 742, 6 (17%) ST97, four (11%) ST1, two (6%) ST30, and two (6%) ST126 (Table 1). Moreover, the PVL genes were most frequently detected in the ST742 lineage MRSA in a total of 14% (3/21). MRSA isolates harboring the *mecC* gene were assigned in the ST742.

According to phylogenetic analyses, ST 742 and ST97 form a cluster, differing only in the *aroE* gene. These STs are phylogenetically close to ST126 with differences in *aroE* gene. Interestingly, the ST352 found in milk samples from Portugal also aligned close to the ST742 and ST97, differing only in the *aroE* gene (Fig. 1).

In Brazil, there are only few studies identifying cases of bovine mastitis caused by MRSA. Recently, Guimarães et al. (2017) reported an outbreak of intramammary infections associated to MRSA in São Paulo [17]. In the present study, we identified a high percentage of MRSA (26%) in bovine



	arcC	*aroE*	*glpF*	*gmk*	*pta*	*tpi*	*yqil*	*CC*
ST 126	3	78	1	1	1	5	3	CC97
ST 352	3	68	1	4	1	5	40	CC97
ST 97	3	1	1	1	1	5	3	CC97
ST 742	3	131	1	1	1	5	3	CC97

Fig. 1 Maximum likelihood phylogenetic tree of *Staphylococcus aureus* STs: In red, isolates from this study. In green, other Brazilian isolates. In blue, China isolates. In pink: isolates from Portugal. The STs are accessed at PubMLST.org: Public databases for molecular typing https://pubmlst.org/ Multilocus sequence typing (MLST) databases and software

subclinical mastitis. This is the first report of *mecC*-positive LA-MRSA isolates in Southeastern Brazil. Just recently, Silva and co-workers reported the occurrence of a LA-MRSA ST126 harboring the *mecC* variant in the North of Brazil [18]. MRSA harboring *mecC* has already been identified in milk samples from animal origin in previous studies elsewhere [7, 12, 19, 20]. Notably, all MRSA isolates harboring the *mecC* gene in the present study did not express resistance phenotype to oxacillin in the vitro testing (OS-MRSA—oxacillin-susceptible *mecC*-positive *S. aureus*). In addition, the *mecC* isolates were also susceptible to cefoxitin disks. Although we did not test that, cefoxitin-agar screening plates might be a more suitable method for detecting these *mecC* isolates. In spite of that, from these and other studies, it is clear that a PCR-based method to detect *mecC* gene is required [21].

The sequence types identified among the *mecC* isolates studied were also rarely found. MRSA belonging to ST97 had also been identified in milk samples from bovine mastitis in China [22] and Tunisia [23] and also from pigs in Japan [24]. Yet, all MRSA isolates from these studies carried the *mecA* gene, while in our study, ST97 harboring *mec*C was for the first time identified. In Brazil, only few studies reported MRSA ST398 [25, 26], ST126, and ST133 [27]. Besides ST97, we identified *mecC* in a ST746 strain, and to the best of our knowledge, a *mecC* ST746 has never been reported before. Interestingly, the MRSA ST746 is genetically similar to ST97, varying in the *aroE* gene. Only recently, Silva and co-workers identified an MRSA ST126 harboring the *mecC* gene [18]. We identify ST126 among the MSSA isolates, but not among the *mecC* MRSA detected. Our report, in addition to the study by Silva and collaborators [18], may indicate a more widely spread of *mecC* among livestock-associated *S. aureus* in Brazil. Future studies are needed to investigate the extent of this change in *S. aureus* epidemiology, the animal management, and the potential dissemination of *mecC* strains in humans, in order to prevent public health and economic impacts. Although we have studied a limited number of milk samples, our data reinforces the entrance of the *mecC* LA-MRSA in Brazil and the need to include *mecC* primers when searching for MRSA in clinical specimens from animal and human origins.

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Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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