Antimicrobial resistance profiles of *Staphylococcus aureus* clusters on small dairy farms in southern Brazil[1]

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ABSTRACT.- Girardini L.K., Paim D.S., Ausani T.C., Lopes G.V., Pellegrini D.C.P., Brito M.A.V.P. & Cardoso M. 2016. **Antimicrobial resistance profiles of *Staphylococcus aureus* clusters on small dairy farms in southern Brazil.** *Pesquisa Vetrinária Brasileira 36(10):951-956.* Departamento de Medicina Veterinária Preventiva, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9090, Porto Alegre, RS 91540-000, Brazil. E-mail: mcardoso@ufrgs.br

In intensive dairy farming, persistent intramammary infection has been associated with specific *Staphylococcus (S.) aureus* strains, and these strains may be resistant to antimicrobials. The objective of this study was to evaluate the antimicrobial resistance phenotypes of *S. aureus* isolates and to assess the distribution and the persistence of clonal groups in small dairy herds of southern Brazil. Milk samples were collected from all lactating cows from 21 dairy farms over a two-year period, totaling 1,060 samples. *S. aureus* isolates were tested for susceptibility to thirteen antimicrobials using the disk diffusion method. The total DNA of the isolates was subjected to SmaI digestion followed by pulsed-field gel electrophoresis (PFGE). Banding patterns differing by ≤4 bands were considered members of a single PFGE cluster. The frequency of *S. aureus* isolation ranged from 3.45% to 70.59% among the 17 *S. aureus*-positive herds. Most *S. aureus* isolates (87.1%) were susceptible to all antimicrobials; resistance to penicillin (18.2%) was the most frequently observed. The 122 isolates subjected to macrorestriction analysis were classified into 30 PFGE-clusters. Among them, only 10 clusters were intermittent or persistent over the two-year period. The majority (93.6%) of isolates belonging to persistent and intermittent clusters were susceptible to all tested antimicrobials. *S. aureus* intramammary colonization in small dairy farms of southern Brazil is most frequently caused by sporadic PFGE clusters, although some persistent clusters can arise over time. Both sporadic and persistent isolates were highly susceptible to antimicrobials.

INDEX TERMS: Mastitis, PFGE clusters, Methicillin-resistant *Staphylococcus aureus,* MRSA.

RESUMO.- [**Perfil de resistência a antimicrobianos de grupos clonais de *Staphylococcus aureus* isolados de pequenas propriedades leiteiras do sul do Brasil.**] A infecção intramamária persistente em bovinos leiteiros tem sido associada com estirpes de *Staphylococcus (S.) aureus* específicos, os quais podem ser resistentes a antimicrobianos. Os objetivos deste estudo foram avaliar os fenótipos de resistência aos antimicrobianos de isolados de *S. aureus* e a distribuição e persistência de grupos clonais em pequenos rebanhos leiteiros do sul do Brasil. As amostras de leite foram coletadas de todas as vacas em lactação de 21 propriedades leiteiras, ao longo de um período de dois anos, perfazendo um total de 1.060 amostras. Isolados de *S. aureus* foram testados quanto à resistência frente a treze antimicrobianos, pelo método de disco-difusão. O DNA total dos isolados foi clivado com a enzima SmaI e submetido a eletroforese em gel de campo pulsado (PFGE). Padrões

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de bandas diferentes por ≤4 bandas foram considerados como pertencentes ao mesmo grupo clonal. A freqüência de *S. aureus* variou de 3,45% até 70,59%, entre os 17 rebanhos com isolamento positivo de *S. aureus*. A maioria dos isolados de *S. aureus* (87,1%) foi suscetível a todos os antimicrobianos; resistência à penicilina (18,2%) foi a mais freqüentemente observada. Os 122 isolados submetidos à análise de macrorestrição foram classificados em 30 grupos clonais de PFGE. Entre eles, apenas dez grupos clonais foram intermitentes ou persistentes ao longo do período de dois anos. A maioria (93,6%) dos isolados pertencentes a grupos clonais persistentes e intermitentes foram suscetíveis a todos os antimicrobianos testados. Concluiu-se que a colonização intramamária em bovinos de pequenas propriedades leiteiras do Sul do Brasil é mais frequentemente causada por grupos clonais esporádicos de *S. aureus*, embora alguns grupos clonais persistentes possam ocorrer ao longo do tempo. Em ambos os grupos clonais os isolados foram majoritariamente suscetíveis a antimicrobianos.

TERMOS DE INDEXAÇÃO: Mastite, grupos clonais, PFGE, *Staphylococcus aureus* resistente à meticilina, MRSA.

INTRODUCTION

Staphylococcus (S.) aureus is the main coagulase-positive species of *Staphylococci* associated with intramammary infections in dairy cattle and can lead to clinical or subclinical mastitis (Young et al. 2001). Although *S. aureus* can colonize the skin and mucous membranes of cows, the udder is the most important reservoir of these bacteria (Capurro et al. 2010). It was demonstrated that *S. aureus* intramammary infection can be widespread among cows within the same herd, and genotyping has shown that common clonal groups can be persistent in a herd over time (Mork et al. 2012, Piccinini et al. 2012). Among the genotyping techniques, pulsed-field gel electrophoresis has been frequently used to determine whether *S.aureus* isolates are close related and associated with persistent intramammary infection (Middleton et al. 2002, Haveri et al. 2008, Mork et al. 2012).

Antimicrobial resistance in *S. aureus* is a concern for both human and animal health, because multi-resistant strains represent a challenge to effective treatment. Particularly, methicillin-resistant *S. aureus* (MRSA), which are resistant to almost all types of β-lactam antimicrobials, have been a concern worldwide (Lee 2003, Van Duijkeren et al. 2004). The presence of MRSA in dairy cows has been investigated because it was demonstrated that cattle may serve as a source of emergence of new MRSA strains in humans (Juhász-Kaszanyitzky et al. 2007, Vanderhaeghen et al. 2010). Therefore, colonization of dairy cattle with antimicrobial-resistant *S. aureus* impacts milk production and may additionally represent an infection hazard to people who work in close contact with cows or consume raw milk (Juhász-Kaszanyitzky et al. 2007).

In southern Brazil, small dairy farms based on family labor contribute a large amount of the total milk production. These independent farmers usually deliver the milk production to processing plants; however, some of the milk is also consumed by the family. One major challenge for small dairy farm production is the lack of technical assistance, leading to failures in animal management and control measures to prevent udder infection (Stumpf et al. 2000). Often, farmers treat animals without any previous knowledge of antimicrobial susceptibility. These practices may influence pathogen transmission and resistance, resulting in an epidemiological scenario quite different from that of dairy farms with large-scale production. Therefore, the aim of this study was to assess the antimicrobial-resistance profiles and pulsotypes of *S. aureus* isolates causing intramammary infection in small dairy herds.

MATERIALS AND METHODS

Herds and sampling. The study population consisted of 1,185 small dairy herds located in a region of approximately 4,821 square kilometers of the state of Rio Grande do Sul, Brazil (UTM zone 22S, 6.69.000N, 350.000E, 6.830.000N, 450.000E). First, farms were stratified according to the average number of cows in lactation (≤10, 11-15, 16-20, 21-25 and ≥25). Next, the sample size was calculated, taking into account an expected *S. aureus* isolation frequency of 20% (Brito et al. 2001), with a precision of 10% at the 95% confidence level. The number of farms of each stratum to be included in the study was determined such that their percent representation in the total target population was maintained (80% of farms had ≤15 cows in lactation). In each stratum, farms were randomly selected for sampling using Microsoft Excel 2010 software. In the 21 farms included in the study, an average of 13 cows (minimum 3 and maximum 50) was in lactation. All sampled farms used mechanical milking and washed the udder before milking. Most farms applied post-dipping treatment with iodine, although no pre-dipping protocol was used. On all farms, clinical mastitis was treated without prior antimicrobial resistance testing.

Samples were collected every six months over a two-year period on all farms. During each sampling event, milk samples were collected from all lactating cows in the herd. Animals that were receiving antimicrobial treatment were excluded from sampling. The procedures for the collection and transport of milk samples followed the recommendations of the National Mastitis Council (2004). Milk samples were collected before the milking process began. The teats were washed and dried individually with disposable paper towels before sampling. The first three streams of milk were discarded, and the teats were then disinfected with 70% alcohol. A composite sample of milk from all teats of each cow was collected in one sterile screw-cap flask. Samples were transported under refrigeration to the laboratory for processing.

Bacterial isolation and identification. Ten microliters of each milk sample was streaked onto 5% sheep blood agar and incubated at 37°C for 24 - 48 hours. After incubation, predominant colonies (at least three colonies present on the agar plate) with similar morphology were isolated and identified as described previously by the National Mastitis Council (2004) and Markey et al. (2013). Gram positive cocci with positive catalase and coagulase test results that displayed acetoin production (Voges-Proskauer test) were classified as *Staphylococcus aureus*.

Antimicrobial susceptibility testing and detection of MRSA. Isolates of *S. aureus* were tested for antimicrobial susceptibility using the disk diffusion test on Müller-Hinton agar (Oxoid, Thermo Scientific, UK) according to the guidelines of the Clinical and Laboratory Standards Institute (2008). The following antimicrobial disks (Oxoid, Thermo Scientific, UK) were tested: cephalothin (30μg), ceftiofur (30μg), clindamycin (2μg), enrofloxacin (5μg), gentamicin (10μg), oxacillin (10μg), penicillin (10 IU), sul-

fonamide (300µg), sulfa + trimethoprim (25µg) and tetracycline (30µg). *S. aureus* ATCC 25923 was used as the quality control. Isolates displaying inhibition zones ≥29mm to penicillin were subjected to the zone edge evaluation of the penicillin disk-diffusion test as recommended (CLSI 2009). Fuzzy zone edges were considered as indicative of no beta-lactamase production.

Isolates that were found to be resistant to oxacillin by the disk diffusion test were subjected to minimum inhibitory concentration (MIC) determination, screened by the Etest® (bioMérieux, Marcy l'Etoile, Lyon, France), and checked for the presence of the *mecA* gene by PCR, as described by Murakami et al. (1991). DNA was extracted after disrupting the bacterial cell wall with Guanidine EDTA-Sarkosyl (Rademaker et al. 1997). The PCR reactions were performed in a total volume of 50 µl composed of 45µL of PCR MasterMix, 1 µL of each primer (*mecA* F: AAA TCG ATG GTA AAG GTT GGC; *mecA* R: AGT TCT GCA GTA CCG GAT TTG C) and 3 µL of template DNA. Amplification was performed for 40 cycles as follows: initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 3 min with a final extension at 72°C for 5 min. Ten microliters of PCR product was analyzed using 2% agarose gel electrophoresis. The reactions were performed in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). *S. aureus* ATCC 25923 was used as a negative control for the *mecA* gene. A *mecA*-positive strain of *S. epidermidis*, which was previously confirmed by sequencing of the *mecA* gene, was used as a positive control (Santos et al. 2015).

Macrorestriction of the total DNA and pulsed-field gel electrophoresis (PFGE). One isolate of *S. aureus* from each *S. aureus*-positive cow was submitted to macrorestriction and PFGE analysis, according to a protocol previously proposed by McDougal et al. (2003). Initially, a single colony of the isolates was inoculated into 5mL of brain heart infusion broth and incubated at 37°C for 24h. The concentrations of the cells were adjusted to an absorbance at 610nm of 0.9 to 1.1 using a spectrophotometer. Two hundred-microliter aliquots of the adjusted cell suspension were centrifuged. The pellet was resuspended in 300µl of Tris-EDTA (TE) buffer (10 mM Tris HCl, 1 mM EDTA [pH 8.0]) and incubated in a water bath at 37°C for 10 min. Three hundred-microliter aliquots of 1.8% of agarose were added to the cell suspension, gently mixed, and dispensed into wells of a plug mold. DNA-soaked agarose plugs were submitted to treatment with 4µL lysostaphin (no. L-7386: Sigma, 1mg / mL). The solidified plugs were transferred to 3 mL of buffer lysis solution (6mM Tris-HCl, 1 M NaCl, 100mM EDTA, 0.5% Brij-58, 0.2% sodium deoxycholate, and 0.5% sodium-lauryl-sarcosine) and incubated at 37°C in a water bath with stirring for 4h or overnight. The plugs were washed four times with 4mL of TE buffer for 10-15 min each and stored at 4°C.

Next, a DNA-plug portion was subjected to digestion with 20 U of the enzyme SmaI (Invitrogen, Carlsbad, CA, USA) for 2-3 hours at 25°C. Electrophoresis was performed with a 1% agarose gel using 0.5X Tris-borate-EDTA buffer with a CHEF DR-II system (BioRad Laboratories, Hercules, CA) at 6 V/cm for 20h at 14°C, with an initial switching time of 5 seconds and a final switching time of 40 seconds. *Salmonella* Braenderup (ATCC# BAA-664) was included as a size reference. After PFGE, the gel was stained with ethidium bromide (2µg/mL, Sigma, St. Louis, USA) for 20 min in a covered container and destained in distilled water for 45 min. The gels were photographed and documented using a Kodak 2200 system (Rochester, New York, USA).

Banding patterns were compared using the Gel-Compar II software package (Applied Maths, Kortrijk, Belgium). Percent similarities between isolate fingerprints were determined on the basis of the Dice correlation coefficient. A band position tolerance of 1% was used for the analysis of PFGE patterns. Dendrograms were generated by unweighted pairwise grouping with mathematical averaging (UPGMA). Isolates were considered as belonging to a common cluster when the PFGE pattern differed by ≤ 4 bands. A cluster was considered transient when isolated only once over a two-year period in a dairy farm; intermittent when it was isolated in more than one sampling event; and persistent when isolated at two or more subsequent samplings.

RESULTS

From 1,060 milk samples collected, 395 (37.2%) tested negative for bacterial growth. *Staphylococcus aureus* was isolated from 136 samples (12.8%), and other *Staphylococcus* spp. were present in 290 samples (27.3%) (Table 1). Other microorganisms (*Corynebacterium* sp., *Streptococcus* sp., *Enterococcus* sp., *Nocardia* sp., *Trueperella pyogenes*, *Escherichia coli, Klebsiella* sp. and yeast) were found in 239 samples (22.5%). On 17 farms, *S. aureus* was isolated at frequencies ranging from 2.6 to 52.9% of the samples; on 14 farms *S. aureus* isolates were obtained in two or more samplings; and five farms were positive in all four samplings.

Among the 132 isolates of *S. aureus* tested by the disk diffusion method, the majority (87.1%; 115/132) were susceptible to all antimicrobial agents. Resistance was detected to penicillin (18.2%; 16/132), tetracycline (2.3%; 3/132), and enrofloxacin (0.7%; 1/132). All penicillin susceptible isolates (n=116) displayed a fuzzy edge zone on the disk diffusion test and were considered as no beta-lactamase producers. All strains were susceptible to cephalothin, ceftiofur, clindamycin, gentamicin, sulfonamide, and sulfa + trimethoprim. Regarding methicillin-resistance, three isolates (2.3%) were resistant in the disk diffusion test to oxacillin. The MICs presented by these strains ranged from

Table 1. Bacteriological test results from milk samples collected on small dairy farms in southern Brazil

Farm	Lactating cows	Milk samples				
		Total	Negative	*S. aureus*	Other *Staphylococci*	Other microorganisms*
A	4	15	4	1	6	4
B	19	76	26	2	31	17
C	15	60	21	4	15	20
D	3	12	4	2	2	4
E	18	70	30	14	7	19
F	7	27	5	4	12	6
G	50	201	98	16	53	34
H	8	31	5	3	17	6
I	11	45	12	10	11	12
J	27	109	51	2	30	26
K	14	54	20	24	9	1
L	15	59	13	6	12	28
M	8	17	3	9	3	2
N	7	26	16	3	2	5
O	7	30	16	3	5	6
P	8	31	6	9	11	5
Q	18	70	17	24	18	11
R	8	31	10	0	11	10
S	12	49	16	0	23	10
T	7	15	10	0	1	4
U	8	32	12	0	11	9
Total		1,060 (100%)	395 (37.2%)	136 (27.35%)	290 (12.83%)	239 (22.54%)

*_Corynebacterium_ sp., _Streptococcus_ sp., _Enterococcus_ sp., _Nocardia_ sp., _Trueperella pyogenes_, _Escherichia coli_, _Klebsiella_ sp. and yeast.

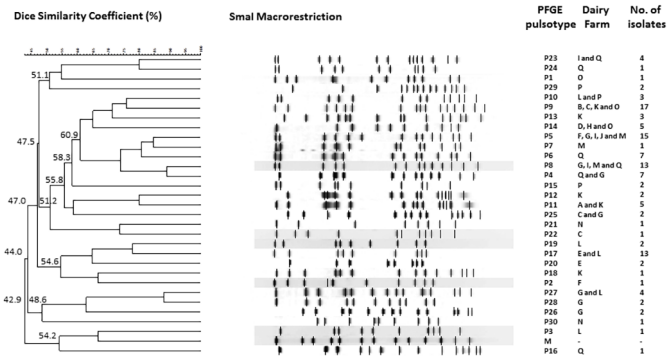

Fig.1. Dendrogram representing the similarity grouping of *Staphylococcus aureus* clusters. The total DNA of isolates was digested with SmaI and separated by pulsed-field gel electrophoresis (PFGE).

0.19 to 0.75μg.mL^{-1} in the Etest (resistance ≥4μg.mL^{-1}). All three isolates were also negative for *mecA* detection and, therefore, were considered methicillin-susceptible.

The most prevalent resistance profile included resistance only to penicillin and was observed in 14 isolates (10.6%). Only one isolate was resistant to antimicrobials belonging to three different classes of antimicrobial agents (penicillin, enrofloxacin and tetracycline) and was thus considered multi-resistant.

Among the 122 *S. aureus* isolates digested by SmaI, 102 different PFGE banding patterns were obtained. The patterns presented between 6 and 16 bands ranging from 33.3 to ~1140 kb. Fourteen patterns were found in more than one isolate (from two to five); the remaining patterns were represented by single isolates. The 102 PFGE banding patterns were grouped into 30 clusters of patterns that differed by ≤4 bands (Fig.1). Four clusters (P5, P8, P9 and P17) encompassed almost the half of the *S. aureus* isolates. Ten clusters (P4, P5, P6, P8, P9, P14, P17, P19, P26 and P27) were found in more than one sampling event at the same respective farms and were classified as intermittent or persistent (Table 2). These clusters encompassed 63 (51.6%) of the *S. aureus* isolates submitted to PFGE profiling. On one farm (K), the same cluster (P9) was persistent over the two-year period of testing. On two farms (Q and G), up to three persistent or intermittent clusters were found concomitantly infecting different cows. Among the isolates belonging to persistent and intermittent clusters, the great majority (59/63; 93.6%) were susceptible to all tested antimicrobials.

Table 2. Distribution of persistent and intermittent *S. aureus* PFGE-pulsotypes among dairy farms sampled in a two-year period in southern Brazil

Dairy farm	Pulsotype [# Sampling event (number of isolates)]
C	P9 [I(1); III(1)]
E	P17 [I(2); II(7); IV(3)]
F	P5 [I(1); II(2)]
G	P5 [I(1); II(2)]; P26 [II(1); III(1)]; P27 [I(2); IV(1)]
H	P14 [I(2)*; II(1)*]
K	P9 [I(4); II(2); III(1); IV(6)]
L	P19 [I(1); IV(1)]
Q	P4 [I(1)*; III(5)]; P6 [II(4); III(3)]

*Isolate displaying resistance to penicillin. All other isolates were susceptible to all tested antimicrobials.

DISCUSSION

In this study, milk of lactating cows with and without symptoms of mastitis was sampled during a two-year period in order to assess the presence of persistent *Staphylococcus aureus* strains causing intramammary infection in small dairy herds in southern Brazil. In 62.3% (665/1060) of the samples, at least one bacterial species was isolated, and among them the genus *Staphylococci* was the most frequent (426/665, 64.1%). The colonization of the udder with coagulase-negative staphylococci is frequently reported in dairy herds, and it may cause mild subclinical mastitis (Mendonça et al. 2012, Mork et al. 2012). In our study, the cows were not examined for alterations in the mammary gland or the milk somatic cell counts were assessed; therefore, it is not possible to draw any conclusion about the implications of the coagulase-negative staphylococci isolation for the udder's health of the sampled animals. On the contrary, the isolation of *S. aureus* from 12.8% (136/1060) of the milk samples from lactating cows, belonging to 17 (17/21, 80.9%) of those farms, demonstrates the relevance of this pathogen in the region. Among the 17 positive herds, in 14 (82.3%) *S. aureus* was isolated in two or more sampling events indicating that *S. aureus* strains were circulating among the animals over time. *Staphylococcus aureus* is a frequent cause of clinical and subclinical mastitis, and chronically infected cows in a herd are an important reservoir of this pathogen (Ericsson et al. 2009, Mork et al. 2012). Considering the highly contagious nature of the infection, the presence of infected cows may constitute a serious risk of the perpetuation and spread of *S. aureus* to susceptible animals and the contamination of milk. Although the inability to eradicate *S. aureus* from dairy herds may be related to failures in standard milking time hygiene as well as in dry cow mastitis therapy (Hutton et al. 1990), other studies raised the hypothesis that certain strains could be more difficult to eliminate from the udder (Smith et al. 1998).

Early attempts for discrimination of *S. aureus* strains were based on typing methods, such as ribotyping and phagotyping, and resulted in the identification of common strains in different herds and geographical areas (Mathews et al. 1994, Aarestrup et al. 1997). Later on, PFGE started to be considered the most discriminatory tool to resolve clonal relationship and proved to be superior to other tested techniques (Tennover et al. 1994, Olive & Bean 1999). Using PFGE, it was demonstrated that *S. aureus* strains are more likely to be unique to a herd than to be found in multiple herds, and once introduced in a herd these strains may become persistent (Joo et al. 2001, Mork et al. 2012). In our study, the 122 *S. aureus* isolates digested by SmaI could be grouped into 30 PFGE clusters, among which three (P5, P8 and P9) encompassed strains originated from four to five different herds. Seven clusters included strains present in two herds and the other 20 clusters were found in only one herd. In this sense, our results are in accordance with the conclusion of Joo et al. (2001) that *S. aureus* strains are

more likely to be associated with the herd than to be widespread among multiple herds in a region.

Regarding the persistence of clusters, ten were considered persistent or intermittent in the farms over the two-year period. Among them, seven clusters were found at least once in up to five herds and two of them (P5 and P9) proved to be intermittent or persistent in two herds each. Although those clonal groups represent one third of the total of clusters, they are distributed among herds as well as persistent. Other studies also pointed out that some clonal groups can cause persistent intramammary infections over months to years (Aarestrup et al. 1995, Buzzola et al. 2001). Since they persist in the udder, they are secreted over time and have a higher potential for spreading. Persistent strains most likely have properties that make them particularly fit to survive in the udder. Genes encoding virulence factors, or those required for biofilm formation, have been reported in persistent strains isolated from the udders of cows (Cucarella et al. 2004, Haveri et al. 2008, Piccinini et al. 2012). Another possible explanation for persistence of clonal groups could be the repeated treatment with antimicrobials, which would lead to the elimination of susceptible strains but allow for the persistence of resistant strains (Rajala-Schultz et al. 2009).

In our study, antimicrobial resistance was not frequent among the *S. aureus* strains, and the majority (87.1%) of the *S. aureus* isolates, including the persistent and intermittent isolates, was susceptible to all tested antimicrobials. Even the resistance to penicillin, which is highly prevalent among *S. aureus* strains in Brazilian herds (Medeiros et al. 2009, Silva et al. 2012), was found in only 18.2% of strains. Among the sampled farms, only clinical mastitis cases were treated with antimicrobials and no susceptibility test was performed. In Brazilian herds, a higher chance of *S. aureus* resistant to penicillin and ampicillin was found in farms that did not perform microbiological cultures and susceptibility tests (Beuron et al. 2014). In the aforementioned study, herds with a higher number of lactating cows (from 10 to >60) were sampled, while in our study 80% of the sampled farms had less than 15 cows in lactation. The size of herds was found to influence the adoption of culture tests, and large herds were found to perform test more often than small herds. In fact, most herds of our study were small and didn't perform culture tests, which may explain the lack of association of antimicrobial resistance and none antimicrobial susceptibility testing. Another study (Pol & Ruegg 2007) pointed out that the higher resistance to penicillin in *S. aureus* strains was attributed to the adoption of dry cow treatment and the long-term exposure to antimicrobials. However, in our study the low frequency of resistant strains cannot be related to the dry cow therapy, which, according to the farmers, was seldom adopted in those herds. The costs of laboratory diagnosis and treatment of chronically infected animals are often not affordable in small herds, and antimicrobial treatment is usually performed only in clinically affected animals. On the other hand, the lack of subclinical mastitis diagnosis may contribute to the persistence of udder infection with susceptible isolates, which could have been eliminated by antimicrobial treatment.

Even considering the penicillin susceptible profile of the tested *S. aureus* strains, we screened MRSA strains by the agar diffusion test using oxacillin discs and submitted the positive strains to *mecA* detection and E-test. Although three isolates were positive in the screening test, none of them carried *mecA* and the MIC values were much lower than the CLSI-breakpoint for susceptibility (≤2μg/mL), which may suggest that the strains were in fact not resistant. A variant of *mecA* has been described (Cuny et al. 2011) and identified in bovine mastitis and in humans (García-Álvarez et al. 2011). However, our isolates presented a different phenotypic resistance profile of strains carrying variant *mecA*, which were resistant to oxacillin in the screening test and displayed high MIC values.

In summary, although persistent clusters were present, a characteristic typically reported in intensive production systems, the predominance of sporadic PFGE clusters demonstrates the wide variability of *S. aureus* found on small dairy farms. This scenario might be related to several factors. Most dairy farmers in the region are not aware of the risks of *S. aureus* colonization to milk production and human health. Furthermore, most small farmers failed to maintain hygiene measures before and after milking, which may facilitate the presence of *S. aureus* on the skin of the udder and its access to the mammary gland. The challenges of *S. aureus* body colonization of lactating cows brought on by their immediate environment have already been demonstrated (Capurro et al. 2010), highlighting the importance of hygiene measures and grouping or culling practices of infected animals in control programs. The lack of these measures on the sampled farms may have been responsible for the new *S. aureus* clusters that were introduced into the mammary gland, as well as for their persistence and continuous circulation among animals or between animals and their environment.

CONCLUSIONS

In summary, the results suggest that *Staphylococcus aureus* intramammary colonization on small dairy farms in southern Brazil is mostly caused by sporadic PFGE-clusters, although some persistent clusters can be present over time.

In both groups, isolates showed to be highly susceptible to antimicrobials.

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