JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Brief Original Article

Molecular identification of coagulase-negative staphylococci isolated from bovine mastitis and detection of β-lactam resistance

Mariela E Srednik[1], Mario A Grieben[2], Adriana Bentancor[1], Elida R Gentilini[1]

[1] *Cátedra de Microbiología, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Buenos Aires, Argentina*
[2] *Cátedra de Bovinos de Leche, Departamento de Producción Animal, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Buenos Aires, Argentina*

Abstract
Introduction: Bovine mastitis is a frequent cause of economic loss in dairy herds. Coagulase-negative staphylococci (CoNS) are increasing in importance as cause of bovine intramammary infection throughout the world in recent years. CoNS have been isolated from milk samples collected from cows with clinical and subclinical mastitis in several countries. Identification of mastitis pathogens is important when selecting appropriate antimicrobial therapy.
Methodology: A total of 93 strains of *Staphylococcus* spp isolated from bovine mastitis in Argentina between 2010 and 2013 were identified by PCR-restriction fragment length polymorphism (PCR-RFLP) using the *gap* gene and were tested for the presence of *bla*Z and *mec*A genes by PCR and for the susceptibility to penicillin and cefoxitin by disk diffusion.
Results: The most common CoNS species was *S. chromogenes* 46.2% (43/93), followed by *S. devriesei* 11.8% (11/93) and *S. haemolyticus* 9.7% (9/93). The *bla*Z gene was detected in 19 (20.4%), but only 16 (17.2%) isolates were resistant to penicillin; the *mec*A was detected in 6 (6.5%) isolates but only 4 (4.3) were resistant to cefoxitin. The 6 *mec*A-positive isolates showed oxacillin MICs ≥ 0.5 µg/ml.
Discussion: CoNS are important minor mastitis pathogens and can be the cause of substantial economic losses. The presence of methicillin resistant isolates emphasizes the importance of identification of CoNS when an intramammary infection is present because of the potential risk of lateral transfer of resistant genes among staphylococcal species.

Key words: bovine mastitis; coagulase-negative staphylococci; molecular identification; antimicrobial resistance; *bla*Z gene; *mec*A gene.

J Infect Dev Ctries 2015; 9(9):1022-1027. doi:10.3855/jidc.5871

(Received 08 September 2014 – Accepted 11 December 2014)

Copyright © 2015 Srednik *et al*. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Bovine mastitis is a disease that affects the health of dairy cows worldwide resulting in decreased milk production and quality [1]. Staphylococci are causing the majority of mastitis cases in different dairy regions around the world. There are differences in pathogenicity between the coagulase-positive and coagulase-negative staphylococcal (CoNS) species. As a group, CoNS have traditionally been considered to be of minor pathogenicity, although in recent years they are increasing in importance as causes of bovine mastitis resulting in tissue damage, decrease in milk production and persistent bovine intramammary infections [2], and have been isolated from milk samples collected from cows with clinical and subclinical mastitis in several countries [3-5] .

Different commercial kits based on specific biochemical reactions often fail in the identification of different species of CoNS [6]. Several genomic targets have been used for the molecular identification of CoNS, including genes for 16S rRNA, *gap*, *hsp*60, *sod*A, *tuf* and *rpo*B [7]. Identification of mastitis pathogens is important to select appropriate antimicrobial therapy. β-lactam antibiotics are frequently used in intramammary therapy. Bacterial resistance to β-lactam mechanisms include production of β-lactamases, encoded by the *bla*Z gene, and production of a low affinity penicillin-binding protein (PBP2a), encoded by the *mec*A gene. The latter, designated as methicillin-resistance (MR) precludes therapy with any of the currently available β-lactam antibiotics [8]. CoNS are reservoirs of resistance genes, potentially threatening public health [9]. MR-CoNS in agricultural animals may act as important reservoirs for the transfer of antimicrobial resistance genes [10]. It has been suggested that *Staphylococcus epidermidis*, after horizontal transfer, facilitates the

potential of S. aureus to resist antibiotic treatment, manifesting in methicillin-resistant *S. aureus* (MRSA).

The aim of this study was to identify CoNS species isolated from mastitic milk of cows and examine genotypic antimicrobial resistance profiles and to compare results obtained by phenotypic and genotypic profiling of resistance to penicillin (PEN) and cefoxitin (FOX), β-lactams antibiotics.

Methodology

CoNS strains

A total number of 93 CoNS were isolated from cows and heifers mastitis samples from different establishments in Buenos Aires, Córdoba, Entre Ríos and Santa Fé. All staphylococci were isolated in the Microbiology laboratory at Facultad de Ciencias Veterinarias, Universidad de Buenos Aires between 2010 and 2013 and maintained frozen at -20°C in tripticase soy broth (Britania Lab. S.R.L, Buenos Aires, Argentina) containing 20% glycerol. Reference strains used in this study were selected from the American Type Culture Collection (ATCC): *S. capitis* ATCC 35661, *S. cohnii subsp cohnii* ATCC 35662, *S. epidermidis* ATCC 12228, *S. haemolyticus* ATCC 29970, *S. saprophyticus* ATCC 15305, *S. sciuri subsp. sciuri* ATCC 29060, *S. simulans* ATCC 27851, *S. warneri* ATCC 49954, *S. xylosus* ATCC 29972.

DNA isolation

CoNS were routinely cultured on brain-heart infusion broth and incubated for 24 hours at 37°C. The whole-cell DNA was isolated using Wizard Genomic DNA purification kit (Promega, Madison, USA).

Species identification by the gap PCR-RFLP

Species identification was achieved by using PCR-restriction fragment length polymorphism (PCR-RFLP). The *gap* gene encoding for glyceraldehydes-3-phospate dehydrogenase, was selected as a target for PCR amplification. PCR amplification tests were performed as in Yugueros *et al*. [19] with some modifications. Briefly, the PCR mixture (50 µl) was composed of 10 µl of 10X PCR amplification buffer, 0.2 mM of dNTPs (Promega, Madison, USA), 0.1 mM of each primer, 1.5 mM Cl_2Mg, 0.5U of *Taq* DNA polymerase (Promega) and 6 µl of DNA extract. The PCR mixtures were subjected to denaturation for 2 minutes at 94°C, followed by 40 cycles of 20 seconds at 94°C, 30 seconds at 60°C, and 40 seconds at 72°C, and a final 5 minutes at 72°C using a termocycler (Eppendorf, Hamburg, Germany). Partial *gap* gene sequencing (Macrogen, Seoul, Korea) was performed for CoNS species as quality control. The amplification products (~931 bp) were digested with 1U of *Alu*I (Fermentas, Waltham, USA) overnight at 37ºC. The resulting DNA fragments were analyzed by 2.5% agarose (Amresco Inc. Irvine, Canada) gel electrophoresis during 2 hours at 70V. To differentiate *S. chromogenes* from *S. intermedius*, PCR products were digested with 1U of *Rsa*I (Fermentas, USA) overnight at 37ºC. For an unambiguous species identification of *S. caprae*, *S. saprophyticus* and *S. xylosus*, PCR products were digested with 1U of *Taq*I (Fermentas, USA) overnight at 65ºC. The same enzyme was used to differentiate *S. sciuri* subsp. *sciuri* and *S. sciuri* subsp. *carnaticus*. To determine an unambiguous species identification of *S. cohnii* subsp. *urealyticus* and *S. devriesei*, the gap gene amplicons were discriminated by digestion with 1U of *Cla*I (Fermentas, USA) overnight at 37ºC.

RFLP in silico

RFLP patterns for the endonucleases *Alu*I, *Cla*I, *Rsa*I and *Taq*I were determined using Restriction Mapper software and the GenBank database for CoNS partial *gap* sequences.

Detection of blaZ and mecA genes

PCR amplifications were performed as previously described (Table 1). Briefly, PCR gene *bla*Z was performed in 50 µl reaction mixture with 10 µl of 10X PCR amplification buffer, 0.2 mM dNTPs (Promega, USA), 0.1 mM of each primer (Ruralex, Buenos Aires, Argentina), 2.5 mM $MgCl_2$, 0.5U of *Taq* DNA

Table 1. Primers for identification and resistance profile of 93 CoNS from bovine milk.

Genes	Primers (5’-3’)	Amplicon size	Reference
gap	5’-ATGGTTTTGGTAGAATTGGTCGTTTA-3’ 5’-GACATTTCGTTATCATACCAACGTG-3’	933 bp[1]	Yugueros *et al*. [24]
*bla*Z	5’-ACTTCAACACCTGCTGCTTTC-3’ 5’-TGACCACTTTTATCAGCAACC-3’	173 bp	Martineau *et al*. [34]
*mec*A	5’-TGGCTATCGTGTCACAATCG-3’ 5’-CTGGAACTTGTTGAGCAGAG-3’	310 bp	Vannuffel *et al*. [35]

[1]The partial *gap* gene sequencing (Macrogen, Korea) was performed for some CoNS species as gold standard test. Sequences were compared with sequences available in GenBank using Basic Local Alignment Search Tool (BLAST) program (data not shown).

polymerase (Promega, USA) and 4 µl DNA extract. The PCR mixture was subjected to the following program: 3 minutes at 96°C, 30 cycles of 30 seconds at 95°C, 30 seconds at 55 °C and 30 seconds at 72°C, and final 4 minutes at 72°C using a termocycler (Eppendorf, Germany). PCR for *mec*A gene was performed in 50 µl reaction mixture with 10 µl of 10X PCR amplification buffer, 0.2 mM of dNTPs 10mM (Promega, USA), 0.1 mM of each primer (Ruralex, Argentina), 3 mM Cl_2Mg, 0.8U of Taq DNA polimerase (Promega, USA) and 6 µl DNA extract. The PCR mixture was subjected to 3 minutes at 93°C, 30 cycles of 60 seconds at 93°C, 60 seconds at 56 °C, and 60 seconds at 72°C, and final 3 minutes at 72°C using a termocycler. PCR products were analyzed by 2% agarose gel electrophoresis (Biodynamics Laboratory Inc., Tokyo, Japan) with 0.5% Tris-borato-EDTA buffer, 10% ethidium bromide staining and UV light observation.

S. aureus ATCC 29213 was used as a positive control and *S. epidermidis* ATCC 12228 as a negative control.

Susceptibility testing

Antimicrobial susceptibility for PEN and FOX were determined *in vitro* using the agar disc diffusion method [21] with 10U PEN disc (BBL) and 30 µg FOX disc (BBL) on Mueller-Hinton agar (Britania, Argentine). Plates were incubated for 24 hours at 35°C before reading diameters. Isolates were categorized as sensitive (S), intermediate (I) or resistant (R) according to CLSI interpretative criteria [21]. The mecA positive isolates were tested for oxacillin (OXA) MIC by E-test strips (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions.

Table 2. Restriction enzymes and restriction fragments size (bp) in each CoNS species identified by PCR-RFLP of the *gap* gene.

CoNS species	***Alu*I**	***Rsa*I**	***Taq*I**	***Cla*I**
S. capitis	327, 198, 132, 79, 72			
S. caprae	327, 237, 138, 102		342, 204, 162, 79, 60	
S. chromogenes	327, 282, 231, 69	266, 251, 249, 165		
S. cohnii subsp. urealyticus[1]	279, 237, 216, 79			671, 179
S. devriesei[1]	279, 240, 216, 79			565, 265
S. epidermidis	240, 198, 150, 138, 102			
S. gallinarum	327, 198, 126, 108, 102			
S. haemolyticus	324, 243, 240, 102			
S. hyicus	327, 225, 153, 135, 69			
S. saprophyticus	327, 243, 135, 102		684, 189, 58	
S. sciuri subsp. sciuri	192, 135, 108, 102, 90		624, 102, 60	
S. simulans	300, 174, 138, 110, 100, 75			
S. warneri	315, 300, 138, 108			
S. xylosus	300, 219, 135, 108, 102		402, 249, 201, 79	

[1]The partial *gap* gene sequences for the two species were determined from isolates in this study and deposited in GenBank before the amplicons digestion in silico with *Cla*I.

Table 3. Genotypic and phenotypic resistance profile of CoNS species identified by PCR-RFLP of the *gap* gene.

Species Identification	**N**	***bla*Z**	**PEN (R)**	**PEN (S)**	***mec*A**	**FOX (R)**	**FOX (S)**	**OXA (R)**
by *gap* PCR-RFLP			(≤28mm)	(>29mm)		(≤24mm)	(>25mm)	≥0.5µg/ml
S. chromogenes	*43*	*6*	*6*		*1*		*1*	*1*
S. devriesei	11							
S. haemolyticus	9	5	5					
S. hyicus	5	1		1				
S. xylosus	5							
S. epidermidis	4	2	2		2	1	1	2
S. warneri	4	4	2	2				
S. cohnii subsp. urealyticus	3				3	3		3
S. simulans	3							
S. saprophyticus	2		1					
S. capitis	1	1	1					
S. caprae	1							
S. gallinarum	1							
S. sciuri subsp. sciuri	1							
Total	93	19	17	3	6	4	2	6

Nucleotide sequence accession numbers

The partial gap gene sequences for two CoNS species isolates from bovine mastitis in this study were deposited in GenBank: *S. cohnii* subsp. *urealyticus* [GenBank:KM251710.1] and *S. devriesei* [GenBank:KM251711.1].

Results

CoNS Identification by the gap PCR-RFLP

The *gap* gene allowed to differentiate 14 species. Table 2 shows the species identified in this study and their PCR-RFLP patterns. All amplicons were digested with *Alu*I. For some species it was necessary to use another endoenzyme due to a difficult differentiation by the *gap* PCR-RFLP when employing only *Alu*I. Table 3 shows the species identified by PCR-RFLP in this study. The most common CoNS species was *S. chromogenes* 46.2% (43/93), followed by *S. devriesei* 11.8% (11/93) and *S. haemolyticus* 9.7% (9/93).

Antimicrobial resistance

According to PCR results (Table 3), 19 CoNS were β-lactam resistant carrying the *bla*Z gene and 6 were MR carrying the *mec*A gene. In three isolates (1 *S. hyicus* and 2 *S. warneri*) resistance to PEN was not observed unless the *bla*Z gene was detected. All MR species had OXA MICs ≥ 0.5 µg/ml, however the cefoxithin zone diameter > 25 mm identified these isolates as OXA susceptible. Correlation between cefoxithin zone diameters and oxacillin MICs for the 6 MR-CoNS was not found. Among *S. chromogenes* strains, the most common CoNS species, β-lactam resistance accounted for 16.3% (7/43), less than in other species. Six of these strains were positive to *bla*Z gene and *mec*A gene was detected in only one strain. Among *S. devriesei* strains, the second most common CoNS species, no β-lactam resistance was observed. For *S. haemolyticus*, the third most common species, β-lactam resistance was more common than in other species reaching 55.6% (5/9). Among the *S. hyicus* strains, *bla*Z gene was detected in only one strain corresponding to 20% (1/5) but no expression to PEN was observed by disk diffusion test. For *S. warneri*, a 100% (4/4) of strains were β-lactam resistant carrying the *bla*Z gene but only 2 strains were resistant to PEN. Among the 4 *S. epidermidis* strains, resistance was observed in 3, one carrying the *bla*Z gene and the other ones carrying the *mec*A gene or both genes. β-lactam resistance was observed in the only *S. capitis* strain. Finally, in one *S. saprophyticus* isolate PEN resistance was observed but no *bla*Z gene was detected.

The species carrying β-lactam resistance more frequently were *S. warneri*, *S. cohnii* subsp. *urealyticus* and *S. capitis* (100%), *S. epidermidis* (75%), *S. haemolyticus*, *S. saprophyticus* (50%).

Discussion

CoNS Identification by the gap RFLP-PCR

A total of 14 CoNS species were identified; the most frequently isolated species was *S. chromogenes* (46.2%) followed by *S. devriesei* (11.8%) and *S. haemolyticus* (9.7%). Comparing to other countries, for what concerns the first most common species our results agree with those reported in Belgium, USA, Netherlands, Switzerland and Germany [11-15]. However, in Sweden [16] the most common species identified was *S. epidermidis*. The second most common species did not agree with other studies. In USA and Netherlands [12,13] *S. epidermidis* was reported as the second most common species, while *S. xylosus* was the second in Belgium and Switzerland [11,14], and *S. simulans* in Germany and Sweden [15,16]. With respect to the third species most frequently identified in our study, our percentages agree with those reported in Switzerland [14], *S. haemolyticus* was reported as the most frequently isolated species in Turkey [17]. Among the least common species identified, they were the same as those reported in other countries [9,11,16,18]. The *S. devriesei* species was identified using the *Cla*I restriction enzyme; we could not identify this species before, staying only within a group. In agreement with Yugueros *et al*. [19], genotypic identification based on *gap* genes is a useful diagnostic tool for the suitable identification of CoNS isolated from animal origin.

Antimicrobial resistance

In this study, β-lactam resistance is low and coincides with Sawant *et al*. [13] in USA but in other countries as Netherlands or Korea the resistance is higher [9,22]. For *S. chromogenes*, the most common CoNS species, resistance to PEN was 13.6%, coinciding with studies in USA and Netherlands [13,9]. For *S. haemolyticus*, the third most common CoNS species, resistance to PEN exhibited 55% not coinciding with other authors; it might be due to the low number of *S. haemolyticus* isolates in this study.

One *bla*Z negative isolate was resistant to PEN, which may be explained by mutations in primer binding sites of resistance genes [23] or by β-lactamase hyper-producing strains [22].

Resistance to PEN was not detected in all isolates carrying the *bla*Z gene, this may be due to the lack of

gene expression without induction, as described for *S. aureus* [20]. From the 6 isolates carrying the *mec*A gene, the resistance to FOX was exhibited in 4. Two isolates were categorized as susceptible to FOX based on the disk diffusion method, but were categorized as resistant to OXA because they showed MICs ≥ 0.5µg/ml. Constitutive presence of *mec*A or its homologues in the absence of phenotypic resistance to oxacillin has been described for *S. sciuri* [24]. Correlation between phenotypic and genotypic resistance sometimes is not found. CLSI guidelines indicate that checking for the presence of *mec*A gene by PCR is the most reliable method for detection of MR [21]. In the current work this profile was detected in 3 species, the most important fact being that the *mec*A gene was found in *S. epidermidis*, a putative zoonotic pathogen [9].

Comparing our results, MR prevalence among CoNS was similar to that found in USA and Switzerland [13,14]. However, CoNS-MR isolated from bovine mastitis is higher in Netherlands while in Korea presents a lower incidence [9,22]. The presence of *mec*A has been detected in various species of staphylococci [13]. *S. epidermidis* and *S. haemolyticus* represents the most frequently observed species among MR-CoNS [15]. However, MR was obtained in *S. epidermidis*, *S.cohnii* subsp. *urealyticus* and *S. chromogenes*.

Multidrug resistance of CoNS can be associated to the presence of β-lactam resistance genes [8]. In this study multidrug resistance (unpublished observations) was not common in *mec*A positive CoNS.

CoNS are a heterogeneous group in which each species had variable antimicrobial resistant rate. Thus, CoNS of mastitis origins should be identified at the species rather than at genus level for effective antimicrobial agent selection. Carriage of antimicrobial resistant genes by CoNS species in cattle may be relevant because it potentially poses a human health hazard.

Acknowledgements

This study was supported by the Secretaría de Ciencia y Técnica, Universidad de Buenos Aires, project W766.

References

1. Bradley A (2002) Bovine mastitis: an evolving disease. Vet J 164: 116-128.
2. Gillespie BE, Headrick SI, Boonyayatra S, Oliver SP (2009) Prevalence of coagulase-negative *Staphylococcus* species from three dairy research herds. Vet Microbiol 134: 65-72.
3. Bradley AJ, Leach KA, Breen JE, Green LE, Green MJ (2007) Survey of the incidence and etiology of mastitis on dairy farms in England and Wales. Vet Rec 160: 253-257.
4. Gentilini E, Denamiel G, Bentancor A, Rebuelto M, Rodriguez Fermepin M, De Torres RA (2002) Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine mastitis in Argentina. J Dairy Sci 85: 1913-1917.
5. Salmon SA, Watts JL, Aerestrup FM, Pankey JW, Yancey Jr RJ (1998) Minimum inhibitory concentrations for selected antimicrobial agents against organisms isolated from mammary glands of dairy heifers in New Zealand and Denmark. J Dairy Sci 81: 570-578.
6. Heikens E, Fleer a, Paauw A, Florijn A, Fluit AC (2005) Comparison of genotypic and phenotypic methods for species-level identification of clinical isolates of coagulase-negative staphylococci. J Clin Microbiol 43: 2286-2290.
7. Ghebremedhin B, Layer F, König W, König B (2008) Genetic classification and distinguishing of *Staphylococcus* species based on different partial *gap*, 16S rRNA, *hsp*60, *rpo*B, *sod*A, and *tuf* gene sequences. J Clin Microbiol 46: 1019-25.
8. Brakstad OG, Maeland JA (1997) Mechanisms of methicillin reistance in staphylococci. APMIS 105: 267-276.
9. Sampimon OC, Lam TJGM, Mevius DJ, Schukken YH, Zadoks RN (2011) Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine milk samples. Vet Microbiol 150: 173-179.
10. Hanssen AM, Kjeldsen G, Sollid JU (2004) Local variants of Staphylococcal cassette chromosome mec in sporadic methicillin-resistant *Staphylococcus aureus* and methicillin – resistant coagulase-negative Staphylococci: evidence of horizontal gene transfer. Antimicrob. Agents Chemother 48: 2285-2296.
11. Supré K, Haesebrouck F, Zadoks RN, Vaneechoutte M, Piepers S, De Vliegher S (2011) Some coagulase-negative *Staphylococcus* species are affecting udder health more than others. J. Dairy Sci 94: 2329-2340.
12. Sampimon OC, Barkema HW, Berends IM, Sol J, Lam TJ (2009) Prevalence and herd-level risk factors for intramammary infection with coagulase-negative staphylococci in Duch dairy herds. Vet Microbiol 134: 37-44.
13. Sawant AA, Gillespie BE, Oliver SP (2009) Antimicrobial susceptibility of coagulase-negative *Staphylococcus* species isolated from bovine milk. Vet. Microbiol. 134: 73-81.
14. Moser A, Stephan R, Ziegler D, Johler S (2013) Species distribution and resistance profiles of coagulase-negative staphylococci isolated from bovine mastitis in Switzerland. Schweiz Arch Tierheilkd 155: 333-338.
15. Febler AT, Billerbeck C, Kadlec K, Schwarz S (2010) Identification and characterization of methicillin-resistant coagulase-neative staphylococci from bovine mastitis. J Antimicrob Chemother 65: 1576-1582.
16. Thorberg BM, Danielsson-Tham ML, Emanuelson U, Persson Waller K (2009) Bovine subclinical mastitis caused by different types of coagulase-negative staphylococci. J Dairy Sci 92: 4962-4970.
17. Bal EB, Bayar S, Bal MA (2010) Antimicrobial susceptibilities of coagulase-negative staphylococci (CNS)

and streptococci from bovine subclinical mastitis cases. . Microbiol 267-274.
18. Kot B, Piechota M, Wolska KM, Frankowska A, Zdunek E, Binek T, Klopotowska E, Antosiewicz M (2012) Phenotypic and genotypic antimicrobial resistance of staphylococci from bovine milk. Pol J Vet Sci 15: 677-683.
19. Yugueros J, Tempprano A, Berzal B, Sánchez M, Hernanz C, Luengo JM, Naharro G (2000) Glyceraldehyde-3-phosphate dehydrogenase-encoding as a useful taxonomic tool for *Staphylococcus* spp. J Clin Microbiol 38: 4351-4355.
20. De Oliveira AP, Watts JL, Salmon SA, Aarestrup FM (2000) Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Europe and the United States. J Dairy Sc. 83: 855-862.
21. Clinical and Laboratory Standards Institute (2013) Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard Fourth Edition and supplement VET01-A4 and VET01-S2 (replaces M31 A3). Clinical Laboratory Standard Institute. Wayne, PA.
22. Moon JS, Lee AR, Kang HM, Lee ES, Kim MN, Park YH, Joo YS, Koo HC (2007) Phenotypic and genetic antibiogram of methicillin-resistant staphylococci isolated from bovine mastitis in Korea. J Dairy Sc. 90: 1176-1185.
23. Haveri M, Suominen S, Rantala L, Honkanen-Buzalsky T, Pyöräla S (2005) Comparison of phenotyping and genotypic detection of penicillin G resistance of *Staphylococcus aureus* isolated from bovine intramammary infection. Vet Microbiol 106: 97-102.
24. Couto I, de Lencastre H, Severina E, Kloos W, Webster JA, Hubner RJ, Sanches IS, Tomasz A (1996) Ubiquitous presence of a *mec*A homologue in natural isolates of *Staphylococcus sciuri*. Microb. Drug Resist 2: 377-391.
25. Martineau F, Picard FJ, Grenier L, Roy PH, Ouellette M, Bergeron MG (2000) Multiplex PCR assay for the detection of clinically relevant antibiotic resistance genes in staphylococci isolated from patients infected after cardiac surgery. The ESPRIT Trial. J. Antimicrob. Chemother. 46: 527-534.
26. Vannuffel P, Laterre PF, Bouger M, Gigi J, Vandercam B, Reynaert M, Gala JL (1998) Rapid and specific molecular identification of methicillin-resistant *Staphylococcus aureus* in endotracheal aspirated from mechanically ventilated patients. J. Clin. Microbiol. 36: 2366-2368.

Corresponding author

Elida R Gentilini
Cátedra de Microbiología, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires. Av. Chorroarín 280 (post code 1427), Buenos Aires, Argentina
Phone: +54-11-4524-8421
Email: egenti@fvet.uba.ar

Conflict of interests: No conflict of interests is declared.