Diversity of staphylococcal species in food producing animals in Spain, with detection of PVL-positive MRSA ST8 (USA300)

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Abstract

This work aimed to determine the prevalence, diversity, antibiotic-resistance phenotype/genotype and virulence factors in staphylococci of farm-animals. Nasal samples of 117 farm-animals (calve: 72; lamb: 37; goat: 8) were collected from one slaughterhouse in La Rioja/Spain and cultured for staphylococci and methicillin-resistant-*Staphylococcus* (MRS) recovery. Identification was performed by MALDI-TOF. Antimicrobial resistance phenotype/genotype was determined by susceptibility testing and specific PCRs. Molecular typing (*spa*-typing, multilocus-sequence-typing, *agr*-typing, SCC*mec*), and detection of 12 virulence genes and human Immune-evasive-cluster (IEC) genes were performed by PCR/sequencing in *S. aureus*. Two marker genes of arginine-catabolic-mobile-element (ACME) were determined by PCR (USA300-MRSA detection). Staphylococci were identified in 50%, 54% and 22% of goat, lamb and calve samples, respectively. Among the 13 *S. aureus* isolates recovered, 11 were susceptible to all antimicrobials tested, and two were multidrug-resistant-MRSA [beta-lactams (*blaZ-mecA*), macrolides [(*msr*(A)/*msr*(B)] and fluoroquinolones]. The MSSA harboured either *tst* or enterotoxin genes, while the MRSA harboured the *lukF/lukS-PV* genes. Five sequence-types were detected. The two MRSA strains (from lamb and goat) were typed as t5173/ST8/agr-I/SCC*mec*-IVa/ACME-positive, corresponding to USA300 clone, and were IEC-B-positive. Among the 47 coagulase-negative-staphylococci (CoNS), six species were identified, predominating *S. simulans* (n=25) and *S. sciuri* (n=11). Fifty-three percent showed resistance to at least one antimicrobial (six multidrug-resistant strains). The following resistance phenotypes/genotypes were detected among CoNS: streptomycin [27.6%; *ant(6)-Ia*, *str*], tetracycline [23.4%; *tet*(M), *tet*(L), *tet*(K)], clindamycin [19.1%; *lnu*(A), *vgaA*], erythromycin [10.6%; *erm*(C), *msr*(A)], chloramphenicol (8.5%; *fexA*), tobramycin (6.4%), penicillin-cefoxitin (4.3%; *blaZ*, *mecA*), and SXT (2.1%). The detection of the MRSA-USA300 lineage in food animals is worrisome and should be further monitored.

Keywords: *Staphylococcus* spp*;* MRSA USA300, PVL; IEC; virulence genes; food producing animals.

1. Introduction

Staphylococcal species are divided in two groups: coagulase-positive staphylococci (CoPS, as *S. aureus*, *S. pseudintermedius* or *S. intermedius*, among others), and coagulase-negative staphylococci (CoNS, as *S. sciuri*, *S. epidermidis*, or *S. saprophyticus*, among others). They generally colonize the skin and mucous membranes of humans and animals (mammals and birds), but are also opportunistic pathogens (Kluytmans, 2010). In humans, staphylococci may be responsible for either skin and soft tissue infections, pneumonia or food intoxication. In livestock, especially among bovines, they cause mastitis, among others (Pantosti, 2012). Staphylococci are also common contaminants of animal-derived foods, such as raw meats or milk-derived products (Lozano et al., 2009; Kluytmans, 2010; Becker et al., 2014). In that context, livestock is a source of staphylococci that can be transmitted to humans, highlighting the need to study and characterize the species from food-producing animals.

Methicillin resistant *S. aureus* (MRSA) of the lineage CC398 is a colonizer of pigs, humans exposed to pig farming and other farm species. MRSA CC398 is also implicated in farm-related infections, and it is therefore designated as livestock associated (LA) MRSA (Benito et al., 2014b; Pantosti, 2012). Furthermore, other *S. aureus* clonal lineages such as CC130, CC599, CC59, CC9, CC133 or CC425 are animal-adapted lineages, causing infections in animals and zoonosis in humans (Gharsa et al., 2012; Cuny et al., 2013; Ben Said et al., 2017). MRSA is of great concern for public health, partly due to its pathogenic potential. *S. aureus* disposes a large variety of virulence factors, such as the staphylococcal enterotoxins, toxic shock syndrome toxin (TSST-1), or Panton-Valentine leucocidin (PVL), among others (Jans et al., 2017). A very pathogenic and invasive MRSA clone globally distributed, named USA300, initially emerged in the USA since 1999 in very crowded-communities, but was then spread to the general population, becoming the major cause of skin and soft-tissue infections (SSTIs) (Glaser et al., 2016; Planet, 2017). MRSA USA300 was associated to the community [Community-Associated (CA)], and combined the following molecular characteristics: multilocus sequence type 8 (ST8), staphylococcal cassette chromosome *mec* type IVa (SCC*mec*IVa) encoding *mec*A, presence of PVL, and presence of the arginine catabolic mobile element (ACME) in 99.9% of the strains (Diep et al., 2008; Planet, 2017). MRSA USA300 clone has also been described as CA-MRSA in few occasions in some European countries, such as Spain, Switzerland or France, while closely related strains have been found in Latin

America (Diep *et al.* 2008; Gómez-Sanz et al., 2013b; Vindel et al. 2014; Jung *et al.* 2016; Planet 2017). Moreover, MRSA USA300 clone was detected in wild boar meat in Germany, and from retail meat in the USA (Kraushaar and Fetsch, 2014; Ge et al., 2017).

Since farm animals are known to be reservoirs for *Staphylococcus* spp., the objective of this work was to determine the prevalence and diversity of staphylococcal species in food producing animals such as calve, lamb and goat, and to study their antimicrobial phenotype/genotype as well as the content in genes encoding virulence factors.

2. Material and methods

2.1 Staphylococcus spp. isolation and identification

Nasal samples of 117 farm animals (calve, 72; lamb, 37; goat, 8) were collected from one slaughterhouse in La Rioja, Spain, during the period of June-September 2009. The calves came from 4 farms, the lambs from 2 farms and the goats from one farm. Samples were inoculated into mannitol-salt-agar and oxacillin-resistance-screening-agar-base for staphylococci and methicillin-resistant-*Staphylococcus* (MRS) recovery, respectively. After incubation at 37ºC for 24h, up to five colonies with different staphylococcal morphology were submitted to identification using the matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (Bruker). For identification of *S. pseudintermedius*, digestion of *pta* gene amplicon with *Mbo*I endonuclease was performed by PCR plus restriction fragment length polymorphism (RFLP) (Bannoehr et al., 2009). All staphylococci with different species (or *spa*-type in case of *S. aureus*) and/or different phenotype of resistance per sample were further characterized.

2.2. Molecular Typing and virulence study

All coagulase-positive staphylococcal (CoPS) strains were typed. In the case of *S. aureus*, *spa*-, *agr*- and SCC*mec*-typing was performed (Benito et al., 2014a). The presence of the *agrD* gene was tested in *S. pseudintermedius* isolates (Bannoehr et al., 2007). Multilocus sequence typing (MLST) was performed for both species. For this purpose, PCR and partial sequencing of seven housekeeping genes for *S. aureus* and *S. pseudintermedius* (www.pubmlst.org) were performed to determine the sequence type (ST) and the clonal complex (CC), as previously described (Enright et al., 2000; Bannoehr et al., 2007; Solyman et al., 2013).

The presence of the genes encoding Panton Valentine Leukocidin (*lukF/lukS-PV*), exfoliative toxins (*eta* and *etb*), toxic shock syndrome toxin (*tst*) and enterotoxins genes (*sea, see, seg, sei, sem, sen, seo, seu*) was studied by PCR for *S. aureus* (Benito et al., 2014a; Hwang et al., 2007). The gene *scn*, belonging to the human Immune Evasive Cluster (IEC), was studied for *S. aureus* isolates and the IEC type was determined for *scn*-positive isolates (Benito et al., 2014b). In addition, the virulence genes for the *S. pseudintermedius* leucocidin (*lukS/F-I*), exfoliative toxins (*siet*, *expA*, *expB*) and enterotoxin (sec_{canine}) were tested for *S. pseudintermedius* isolates (Gharsa et al., 2015).

Specific PCRs were performed to determine the presence of the ACME in the CC8 strains, using the primer pairs AIPS.27 + AIPS.28 and AIPS.45 + AIPS.46 for *arcA*, and *opp3* genes, respectively (Diep et al., 2008). The PCR program used in this study consists of an initial denaturation for 7 min at 94°C, followed by 30 cycles of denaturation for 1 min at 94°C, annealing for 1 min at either 55°C (*arcA*) or 61ºC (*opp3*), and an extension for 1 min at 72°C, and a final extension for 10 min at 72°C.

2.3. *Antibiotic susceptibility testing and antibiotic resistance gene detection*

For all the isolates (CoPS and CoNS), susceptibility to penicillin, cefoxitin (oxacillin in the case of *S. pseudintermedius*), gentamicin, tobramycin, tetracycline, chloramphenicol, erythromycin, clindamycin, ciprofloxacin, linezolid, and trimethoprim/sulfamethoxazole (SXT) was analysed by disk-diffusion method (CLSI, 2018). In addition, streptomycin susceptibility was also tested (CASFM, 2018). The detection of the following antimicrobial resistance genes was performed by PCR, according to the phenotype of resistance: beta-lactams (*blaZ*, *mecA*, and *mecC)*, tetracycline [*tet*(K), *tet*(M), and *tet*(L)], aminoglycosides [*aac*(6')-*aph*(2'')*,* ant(4')-Ia], macrolides-lincosamides [*erm*(A), *erm*(B), *erm*(C), *erm*(T), *msr*(A)*/msr*(B), *lnu*(A), *lnu*(B), *vga*(A)], streptomycin [*str*, *ant*(6)-Ia] trimethoprim (*dfrA*, *dfrD*, *dfrG*, *dfrK*) and phenicols *(*cat_{pc194}, cat_{pc221}, cat_{pc223}, *cfr*, *fexA*, *optrA*, and *poxtA)* (Sutcliffe et al., 1996; Kehrenberg and Schwarz, 2005; Schnellmann et al., 2006; Gómez-Sanz et al., 2013b; Liu et al., 2013; Benito et al., 2014b; Wang et al., 2015; Antonelli et al., 2018).

3. Results

3.1. Detection of staphylococcal species

Staphylococcus spp. (CoPS and CoNS) were detected in 50%, 54% and 21% of goat, lamb and calve samples, respectively. Twelve animals harboured CoPS isolates, and 14 isolates were obtained (one isolate/animal, except for two animals in which two *S. aureus* of different *spa*-types were obtained). In addition, 33 animals harboured CoNS and 47 isolates were recovered (1-5 isolates/sample, with different species or antibiotic resistance phenotypes). Six of the tested animals harboured both CoPS and CoNS isolates.

Thirteen of the 14 CoPS isolates were identified as *S. aureus*, and one isolate as *S. pseudintermedius* (lamb origin). *S. aureus* was recovered of lamb (n=11; 29.7%), goat (n=1; 12.5%) and calve samples (n=1; 1.4%) (Table I). The 47 CoNS isolates (33/117 animals) belonged to six distinct species with predominance of *S. simulans* (n=25) and *S. sciuri* (n=11), followed by *S. cohnii* (n=5), *S. chromogenes* (n=4), *S. epidermidis* (n=1) and *S. hominis* (n=1) (Table II).

3.2. Coagulase positive Staphylococcus: molecular typing, antimicrobial resistance and virulence

Among the 13 *S. aureus* isolates, six *spa*-types were detected, including one novel, registered as t1754, and five STs, two of them being especially relevant: ST8-CC8 (n=2) and ST133/CC133 (n=7) (Table I). The *S. aureus* isolates were all typed *agr*-I, except for one *agr*-IV. Most of the strains (n=11, 84.6%) showed susceptibility to all the antimicrobials tested, while only two (15.4%) showed resistance to some antimicrobials. The nine isolates of the clonal complexes CC133 and CC522 were included among the antimicrobial susceptible strains; they all harboured the virulence gene *tst* and were isolated from lamb samples. The two other antimicrobial susceptible isolates (ST45 and ST2329) hosted enterotoxin genes *(seg, sei, sem, sen, seo, seu)*, while only one was positive for the *scn* gene (IEC type-B) (Table I). The remaining two isolates (recovered from lamb and goat samples) showed resistance to cefoxitin (*mecA*), penicillin (*blaZ*), erythromycin (*msrA*) and ciprofloxacin, and harboured the gene encoding PVL, as well as the *scn* gene (IEC type B). Both MRSA strains were typed as t5173/ST8/CC8/*agr*-I/SCC*mec*IVa, thus, considered as the USA300 clone (Table I). Furthermore, they were positive for the ACME marker genes (*arcA* and *opp3* genes).

The *S. pseudintermedius* strain, recovered from a lamb sample, was resistant to tetracycline [carrying the *tet*(M) gene] and harboured the following virulence genes:

lukS/F-I, siet, expA and *expB.* This strain was typed as *agr*D- II and presented a new MLST allelic combination (*ack*:1; *cpn60*:9; *fdh*:2; *pta*:1; *purA*:8; *sar*:1; *tuf*:1), rendering a new sequence type registered as ST932. The ST932 is a double locus variant of the known ST309.

3.2. Coagulase negative Staphylococcus: antimicrobial resistance phenotype and genotype

The CoNS isolates presented diverse resistance phenotypes and genotypes, which are summarized in Table II. Twenty-five isolates (53%) were susceptible to all the tested antimicrobial agents while twenty-two (47%) were resistant to at least one agent tested. Among the resistant isolates, six strains of the species *S. simulans* (3/25), *S. sciuri* (2/11) and *S. cohnii* (1/5) presented a multidrug resistance phenotype (including at least three antimicrobial families). The following resistance rates were detected among the CoNS isolates: streptomycin (27.6%), tetracycline (23.4%), clindamycin (19.1%), erythromycin (10.6%), chloramphenicol (8.5%), tobramycin (6.4%), penicillin (4.3%), cefoxitin (4.3%), and SXT (2.1%). Streptomycin resistance was mediated by *str* gene for most strains, and *ant(6)-Ia* gene in one case. Resistance to tetracycline was encoded by either *tet*(K) or *tet*(L) genes in the strains from calve, and by *tet*(K) alone or associated to either *tet*(L) or *tet*(M) in the isolates from lamb (Table II). *lnu*(A) gene was detected in 66.6% of the clindamycin-resistant strains, and *vgaA* gene in 22.2% of them. Resistance to erythromycin was mediated by either *erm*(C) or *msr*(A). All the chloramphenicol-resistant strains harboured the *fexA* gene but were negative for the *optrA, poxtA* and *cfr* genes. The penicillin and cefoxitin resistant isolates carried *blaZ* and *mecA* genes, respectively, while SXT-resistant isolates harboured the *dfrK* gene.

4. Discussion

Staphylococcal species are opportunistic pathogens and commensals for humans and animals (Pantosti, 2012). Animal-to-human transmission of bacteria may occur by either contact with the animals or consumption of animal-derived products (Kluytmans, 2010), which can contribute to the spread of resistance genes and pathogenicity factors in different ecosystems. In that context, this work aimed to isolate and characterize strains of *Staphylococcus* spp. from food producing animals.

The prevalence rate of *S. aureus* for lamb (29.7%) was higher than the rates for goat (12.5%) and calve samples (1.4%). A similar pattern but with higher percentages was

observed in a recent similar study performed in Greece (Papadopoulos et al., 2018). Furthermore, one isolate of *S. pseudintermedius* was detected in a lamb sample, although this staphylococcal species is generally associated to dogs (Gómez-Sanz et al., 2013a). This species has been formerly identified in equine and bovine at low rates (Rubin et al., 2011), as well as in wildlife (Mama et al., 2019a).

Molecular typing revealed CC133 as the most frequent clonal complex among *S. aureus* recovered (7/13), always as methicillin-susceptible *S. aureus* (MSSA) isolates. Lineage CC133 is particularly frequent among MSSA from small ruminants such as sheep and goat (Eriksson et al., 2013; Ben Said et al., 2017), as well as from donkeys (Gharsa et al., 2012). In addition, *S. aureus* CC133 has also been found in wildlife in Spain (Mama et al., 2019a). The clonal complex CC522 is commonly observed among ruminants and animal-derived products (Gharsa et al., 2012; Ben Said et al., 2017), was also present among our isolates. All strains belonging to the CC133 and CC522 lineages were obtained from lamb samples and harboured the *tst* gene related to the toxic shock syndrome. This was not surprising as such gene was highly frequent among sheep strains of the same genetic lineages (Ben Said et al., 2017). On the other hand, the presence of a calve strain MSSA/ST45 harbouring the *egc*-like operon genes (*seg-sei-sem-sen-seo-seu*) is relevant. This hospital and/or community-associated clone was detected before in healthy humans (Gómez-Sanz et al., 2013b), and in meat samples in Spain (Benito et al., 2014a). In the last case, the strains were typed as IEC-B, as in our work, suggesting a human origin.

The strains ST8 (n=2), isolated from lamb and goat, were typed as t5173/*agr*I/SCC*mec*IVa, were PVL-positive and ACME-positive, thus designated as clone USA300. The clone USA300 is a CA-MRSA that emerged mainly in the USA, representing the major cause of skin and soft-tissue infections (SSTIs) (Diep et al., 2008; Glaser et al., 2016; Planet, 2017). It was also sporadically found in some European countries, while a similar variant was found in Latin America (Vindel et al., 2014; Jung et al., 2016; Planet, 2017). According to some authors, the CA-MRSA dominant in the USA is the USA300 North American (USA300-NA) MRSA clone, which descended from USA500-like MSSA strains by acquisition of various mobile genetic elements (MGEs) (Glaser et al., 2016). The acquired MGEs include: a) the SCC*mec*IVa element (with *mecA* gene); b) *S. aureus* pathogenicity island 5 containing *sek* and *seq* genes; c) phage phiSA2 carrying the Panton-Valentine leukocidin genes; and d) ACME type I, which are assumed to confer enhance pathogenicity and fitness on USA300-NA MRSA

strains (Diep et al., 2008; Glaser et al., 2016; Planet, 2017). In addition, plasmid analysis showed the presence of the plasmid p18805-p03 encoding diverse antibiotic resistance genes [aminoglycosides, *ant*(6)-Ia and *aph*(3')-III; beta-lactams, *blaZ*; macrolides *mph*(C); macrolides- lincosamides-streptogramin B, *msr*(A)] (Glaser et al., 2016). Our strains correspond to the USA300-NA clone, and the multidrug resistance and the presence of PVL genes, suggest the acquisition of MGEs. Furthermore, the presence of IEC system (type B) confirms the potential adaptation to humans. Unexpectedly, that clone has been found, as in this work, in animals, such as an infected dog in France (Haenni et al., 2012), wild boar meat in Germany (Kraushaar and Fetsch, 2014), and retail meat in the USA (Ge et al., 2017). In those cases, the USA300 strains detected were suspected to be transmitted by humans (hypothetically the dog owner or food handlers). These data are important in the context of the One Health perspective, as it reflects how the dissemination of bacteria with their mobilizable resistance and virulence genes content can occur between the clinic, community, animals and food. Additionally, the presence of *S. pseudintermedius* in a lamb sample may suggest a contact between the animal and dogs or dog owners.

Six species of CoNS were identified among the tested animals (28% were positive): *S. simulans*, *S. sciuri S. cohnii*, *S. chromogenes*, *S. epidermidis* and *S. hominis*. Those species seem to be recurrent in cattle samples (De Visscher et al., 2014; Cuny et al., 2017). CoNS could be a reservoir of clinically-relevant resistance genes that could be transferred to *S. aureus* isolates (Becker et al., 2014). Half of the CoNS strains were resistant to at least one tested antimicrobial agent. The resistance phenotypes observed among our isolates were not unexpected since beta-lactams, tetracyclines, lincosamides and sulphonamides are antimicrobial families very frequently used in the veterinary sector (food-producing animals and pets) (Prestinaci et al., 2015). In addition, the phenotypic and genotypic data were in accordance with a previous study on animal-derived food (Chajecka-Wierzchowska et al., 2015). Alternatively, the *fexA* gene, though uncommon, seems to become increasingly present in CoNS isolated from animals (Mama et al., 2019b). It was first identified in a bovine *S. lentus* isolate (Kehrenberg and Schwarz, 2004), and was later found in other staphylococcal species such as *S. sciuri*, *S. simulans* and *S. aureus* from food-producing animals, equidae and wild boar (Kehrenberg and Schwarz, 2006; Mama et al., 2019b). In this study, the gene was detected in *S. simulans* and *S. chromogenes* strains. Recently, the *fexA* gene has been detected associated with

the *cfr* and *optrA* linezolid resistance genes in the same plasmid (Li et al., 2016), although those genes were absent in our *fexA* positive strains.

Conclusion

Farm animals are reservoir for *Staphylococcus* spp., including CoPS and CoNS carrying relevant resistance genes [*mecA*, *lnu*(A), *vgaA*, *fexA*], and important virulence factors (*lukF/lukS-PV*, *tst*, enterotoxins). The presence of the CA-MRSA-USA300/ACME+/IEC-B in farm animals as well as the CA-MSSA/ST45/ IEC-B is a subject of concern, which opens the question about the origin of the isolates and the transference of virulent staphylococci between different ecosystems.

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Table I: Phenotypic and genotypic characteristics of coagulase positive *Staphylococcus* isolates from different origins

Origin	Species	Number of isolates	ST-CC-SCC*mec*	Spa-type (N° strains)	*agr*	Antimicrobial resistance		Virulence genes	*scn* gene	IEC type
						Phenotype[c]	Genotype			
Lamb	*S. aureus*	7	ST133-CC133	t17754[b] (4)	I	Susceptible	-	*tst*	-	
				t2678 (3)		Susceptible	-	*tst*	-	
	S. aureus	2	ST522-CC522	t1534	I	Susceptible	-	*tst*	-	
	S. aureus	**1**	**ST8-CC8-IVa[a]**	**t5173**	**I**	**PEN-FOX-ERY-CIP**	***blaZ-mecA-msrA***	***lukF/lukS-PV***	**+**	**B**
	S. aureus	1	ST2329	t8765	IV	Susceptible	-	*sei-sem-sen-seu*	-	
	S. pseudintermedius	1	ST932[b]	-	II	TET	*tet*(M)	*lukF/S-I-siet-expA-expB*		
Goat	***S. aureus***	**1**	**ST8-CC8-IVa[a]**	**t5173**	**I**	**PEN-FOX-ERY-CIP**	***blaZ-mecA-msrA***	***lukF/lukS-PV***	**+**	**B**
Calve	*S. aureus*	1	ST45-CC45	t505	I	Susceptible	-	*seg-sei-sem-sen-seo-seu*	+	**B**

[a]strain positive in the ACME specific PCR. Marked in bold letter

[b]new spa-type or new ST

[c]PEN: penicillin; FOX: cefoxitin; TET: tetracycline, ERY: erythromycin, CIP: ciprofloxacin

Table II: Coagulase negative *Staphylococcus* of different origins, phenotypes and genotypes.

Origin (nº of positive animals)	**Species** (nº of isolates)	**Antibiotic resistance phenotype**[a] (nº of isolates)	**Genes detected** (nº of isolates)
Lamb (17)	*S. simulans* (19)	STR (2)	*str* (2)
		STR-TET (3)	*tet*(K) (3) - *tet*(L) (1) - *str* (3)
		TET-SXT (1)	*tet*(K) - *tet*(L) - *dfrK*
		STR-TET-CLOR (1)	*tet*(K) - *tet*(M) - *ant*(6)-*Ia* - *fexA*
		ERY-CLIN-STR-TET-CLOR (1)	*tet*(K) - *tet*(M) - *erm*(C) - *fexA*
		Susceptible (11)	
	S. sciuri (4)	CLIN (1)	*lnu*(A)
		CLIN-STR (1)	*lnu*(A) - *str*
		Susceptible (2)	
	S. cohnii (4)	FOX-STR-TET (1)	*tet*(K) - *tet*(L) - *mecA* - *str*
		Susceptible (3)	
	S. chromogenes (2)	Susceptible (2)	
	S. epidermidis (1)	PEN	*blaZ*
Calve (14)	*S. simulans* (3)	TET (1)	*tet*(K)
		ERY-CLIN-STR-CLOR (1)	*fexA* - *str*
		Susceptible (1)	
	S. sciuri (7)	CLIN-TOB (1)	*lnu*(A) (1)
		TOB-TET (1)	*tet*(K)
		CLIN-TET (1)	*tet*(K) - *lnu*(A)
		FOX-STR-TOB-TET (1)	*tet*(L) - *mecA*
		CLIN-STR-TOB-TET (1)	*tet*(K) - *lnu*(A)
		Susceptible (2)	
	S. cohnii (1)	Susceptible (1)	
	S. chromogenes (2)	ERY-CLIN-CLOR (1)	*erm*(C) - *vgaA* - *fexA*
		ERY-CLIN-STR (1)	*erm*(C) - *vgaA* - *lnu*(A)
	S. hominis (1)	PEN-ERY	*blaZ* - *msrA*
Goat (3)	*S. simulans* (3)	Susceptible (3)	

[a]PEN: penicillin; FOX: cefoxitin; TET: tetracycline; CLIN: clindamycin; ERY: erythromycin; TOB: tobramycin; ClOR: chloramphenicol; SXT: trimethoprim/sulfamethoxazole