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PREVALENCE AND CHARACTERISTICS OF METHICILLIN-RESISTANT STAPHYLOCOCCI IN GOATS ON THE ISLAND OF TENERIFE, SPAIN

Rossana ABREU[1,2], Cristobalina RODRÍGUEZ-ÁLVAREZ[1], María LECUONA[2], Beatriz CASTRO-HERNÁNDEZ[2], Juan Carlos GONZÁLEZ[3], Armando AGUIRRE-JAIME[4] and Ángeles ARIAS[1*]

[1]Department of Preventive Medicine and Public Health, University of La Laguna, Campus de Ofra S/N, Santa Cruz de Tenerife 38071, Spain; [2]University Hospital of the Canary Islands, San Cristobal de La Laguna, Tenerife, Canary Islands, Spain; [3]Canary Islands Health Service, Santa Cruz de Tenerife, Canary Islands, Spain; [4]Institute of Care Research, Nurses Association of Santa Cruz de Tenerife, Spain

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The aim of this study was to determine the prevalence of methicillin-resistant *Staphylococcus* (MRS) in healthy goats on the Island of Tenerife, Spain, as well as to identify the phenotypic and genotypic characteristics of the strains found. A cross-sectional prevalence study was conducted. A total of 158 goats from 15 different farms were sampled between September 2017 and January 2018. The percentage of positive samples of methicillin-resistant *Staphylococcus aureus* (MRSA) was 15.8% (25/158) and that of methicillin-resistant coagulase-negative staphylococci (MRCoNS) was 6.9% (11/158). All MRSA isolates from goats belonged to one clonal group showing Multi-Locus Sequence type 398. All strains studied (n = 36) were resistant to non-carbapenem beta-lactam antibiotics and susceptible to teicoplanin, linezolid, vancomycin, rifampicin, quinupristin-dalfospristin and mupirocine. In MRSA isolates, the highest percentage of resistance obtained, besides beta-lactam non-carbapenem antibiotics, was to trimethoprim-sulphamethoxazole and, in the case of MRCoNS isolates, to phosphomycin and erythromycin. A total of 12 resistance patterns were obtained, presenting differences between patterns obtained for MRSA and MRCoNS, with 7 different patterns for MRSA and 5 for MRCoNS. We therefore consider it essential to expand the epidemiological study of these strains of animal origin, as well as to increase surveillance and control measures at all stages of the food chain.

Key words: Methicillin-resistant *Staphylococcus aureus*, methicillin-resistant coagulase-negative staphylococci, phenotypic and genotypic characteristics, goat

Methicillin resistance in *Staphylococcus aureus* is a serious concern in both human and veterinary medicine (Hernández-Porto et al., 2014; Goerge et al., 2017; Ortwine and Bhavan, 2018). The main gene linked to resistance to the-

*Corresponding author; E-mail: angarias@ull.edu.es; Phone: 0034 (922) 319-369

se antibiotics is the *mecA* gene, found in Staphylococcal Cassette Chromosome mec (*SCCmec*), which are mobile genetic elements responsible for the transfer of resistance genes (Shore and Coleman, 2013) and are also part of the genome of coagulase-negative *Staphylococcus* (MRCoNS) species (Vanderhaeghen et al., 2014; Fernandes Dos Santos et al., 2016). Several studies indicate that, like MRSA, MRCoNS have animal production facilities as a reservoir and can be found in meat products and other foods such as the milk of ruminants (Vanderhaeghen et al., 2014; Landeta et al., 2013; Nemeghaire et al., 2014).

The presence of *Staphylococcus* strains, such as MRSA and MRCoNS, is widely described in mastitis of various ruminants including goats, and, as a consequence, in their milk (Chu et al., 2012; Cortimiglia et al., 2015; Mahato et al., 2017; Obaidat et al., 2018). In recent years, a great number of studies have been carried out on colonisation prevalence of multiresistant *Staphylococcus* strains in different farm and domestic animals in farm environments and in people in contact with them (Habrun et al., 2011; Morcillo et al., 2012; Pletinckx et al., 2013; Sun et al., 2017; Pomba et al., 2017; Rodrigues et al., 2018; Sato et al., 2018; Feld et al., 2018), mostly with reference to MRSA.

To date, however, very few studies have been conducted on the colonisation of multiresistant bacteria in goats (Aquino et al., 2012; Loncaric et al., 2013). Goat production is widespread in the Canary Islands, as it easily adapts to the weather conditions of the islands. The goat production systems are semi-intensive, where the animals graze freely and are kept in stables for the night. As the island of Tenerife is geographically remote from the European and African continents, animals are more isolated than those on the continent.

The objective of this study was to determine the prevalence of methicillin-resistant *Staphylococcus* in healthy goats on the island of Tenerife, Spain, as well as to identify the phenotypic and genotypic characteristics of the isolated strains.

Materials and methods

Collection of samples

A cross-sectional prevalence study was conducted. A total of 158 goats were screened, representing a randomised selection of animals from 15 wean-to-finish farms for local consumption. Goat samples from farms all over the island were collected between September 2017 and January 2018. The study was authorised by the General Public Health Direction of the Health Service of Canary Islands. The sampling was done by a veterinarian of that Public Health Direction. All samples were collected with cotton-tipped swabs that were placed in Amies Rayon (deltalab^{TM}), stored at 4 °C and transported directly to the laboratory.

Isolation and identification of MRS isolates

Nasal swabs were incubated in Brain-Heart Infusion (BHI) with 7% NaCl for 18–24 h at 37 °C. Then, 10 µl of the infusion was plated onto MRSA-ID culture plates (bioMérieux™). The plates were then incubated aerobically at 37 °C for 24–48 h. *Staphylococcus* suspicious colonies were identified by morphology and growth colour. Catalase test and coagulase test agglutination (Slidex™ Staph Plus, bioMérieux™) were performed on malachite green and white coloured colonies. Species identifications were confirmed by the Vitek II Automated Microbiology System with ID card GP (bioMerieux™). Methicillin resistance was confirmed by testing for the presence of penicillin-binding protein A (PBP2a) (MRSA-screen; Denka Seiken Co™, Japan) and the presence of the *mecA* gene was detected by Real-Time PCR (RT-PCR) (iQ™5, BioRad). *Staphylococcus aureus* ATCC 29213 was used as reference strain.

Molecular typing of MRSA

DNA macro-restriction analysis by pulsed-field gel electrophoresis (PFGE). Isolation of chromosomal DNA was performed as described by Smith et al. (1988). The enzyme used for the macro-restriction was ApaI (Promega) with the following electrophoresis conditions: block I: 6 v/cm, 12 h, 5–15 sec; block II: 6 v/cm, 12 h, 15–40 sec. The results were interpreted according to the criteria described by Tenover et al. (1995).

Sequencing. All MRSA isolates were analysed by multilocus sequence typing (MLST) as described previously (Enright et al., 2000). MLST was based on a sequence analysis of the internal fragments of seven housekeeping genes: *arcC* (Carbamate kinase), *aroE* (Shikimate dehydrogenase), *glpF* (Glycerol kinase), *gmk* (Guanylate kinase), *pta* (Phosphate acetyltransferase), *tpi* (Triosephosphate isomerase), *yqi* (Acetyl-coenzyme A acetyltransferase). The allelic profiles and sequence types were assigned according to the *S. aureus* MLST database (https://pubmlst.org/).

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined by the broth microdilution method using Vitek-2 AST-626 cards (bioMérieux®, France). *Staphylococcus aureus* ATCC 29213 was used as reference strain. Strains were tested for susceptibility to beta-lactams: benzylpenicillin (PGL/PG), oxacillin (OXA); aminoglycosides: gentamicin (GM), tobramycin (TM); glycopeptides: teicoplanin (TP), vancomycin (VA); quinolone: levofloxacin (LV); lincosamide: clindamycin (CC); macrolide: erythromycin (E); rifamycin: rifampicin (RI); bacteriostatics: trimethoprim-sulphamethoxazole (SXT), fusidic acid (FA); streptogramins: quinupristin–dalfopristin (QDA); oxazolidinone: linezolid (LZ); glycylcycline: tigecycline (TGC); phosphate: phosphomycin (FM); monocarboxylic acid: mupirocin (MU).

The breakpoints used were the same as those established by the Clinical and Laboratory Standards Institute Guidelines (2011).

Statistical analysis

The characteristics of the samples are described with the relative frequency of the categories that make up nominal variables. Frequency comparisons were performed with Fisher's Exact Test at a significance level $P \leq 0.05$. Calculations were performed with the IBM Co™ statistical processing package for PC in operating environment Windows SPSS 21.0.

Results

The prevalence of methicillin-resistant *Staphylococcus* was 22.8% (36/158), of which 15.8% (25/158) corresponded to MRSA and 6.9% (11/158) to MRCoNS. These latter strains were found through chromogenic medium while detecting MRSA, all being positive for the *mecA* gene. The following species were identified: *Staphylococcus lentus* (n = 3, 27.2%), *Staphylococcus sciuri* (n = 3, 27.2%), *Staphylococcus haemolyticus* (n = 2, 18.2%), *Staphylococcus auricularis* (n = 1, 9.1%), *Staphylococcus gallinarum* (n = 1, 9.1%), *Staphylococcus pasteuri* (n = 1, 9.1%).

Genotypic study of the MRSA strains demonstrated, according to the analysis of the patterns obtained by PFGE, that the isolates were closely related and almost all belonged to the same clone (Fig. 1). MLST confirmed that all isolates belonged to ST398 (CC398).

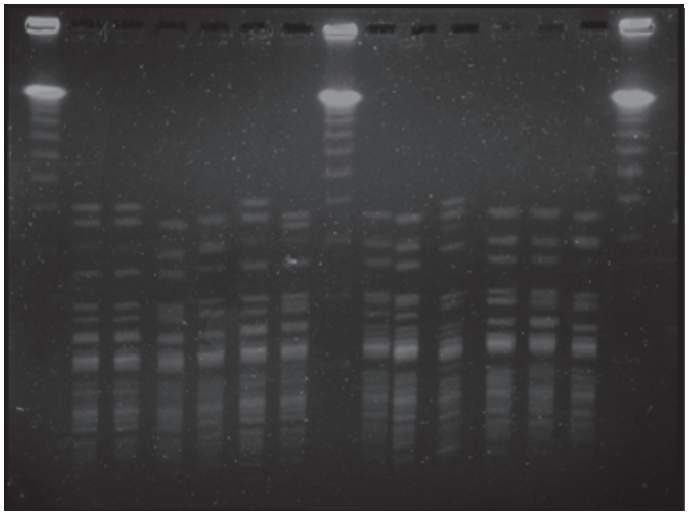

Fig. 1. Molecular typing by PFGE of ApaI-digested DNA from MRSA isolates from goats

All strains studied (n = 36) were resistant to beta-lactam non-carbapenem antibiotics and sensitive to teicoplanin, linezolid, vancomycin, rifampicin, quinupristin-dalfopristin, and mupirocine.

Table 1 shows the antibiotic resistance percentages of the strains tested and their distribution for MRSA and MRCoNS. Besides beta-lactam non-carbapenem antibiotics, the MRSA isolates showed the highest percentage of resistance to trimethoprim-sulphamethoxazole and the MRCoNS isolates to phosphomycin and erythromycin. There were significant differences in resistance to erythromycin (P = 0.006), fusidic acid (P = 0.023), phosphomycin (P = 0.000), and trimethoprim-sulphamethoxazole (P = 0.047).

Table 1

Antimicrobial resistance of *mecA Staphylococcus* isolates from goats in Tenerife, Spain

Antimicrobial	Total n (%)	MRSA n (%)	MRCoNS n (%)	Significance (P)
Benzylpenicillin	36 (100)	25 (100)	11 (100)	–
Oxacillin	36 (100)	25 (100)	11 (100)	–
Gentamicin	4 (10.8)	4 (16.0)	0 (0)	0.290
Tobramycin	4 (10.8)	4 (16.0)	0 (0)	0.290
Clindamycin	9 (24.3)	5 (20.0)	4 (36.4)	0.409
Erythromycin	13 (35.1)	5 (20.0)	8 (72.7)	0.006
Trimethoprim-sulphomethoxazole	19	16 (64.0)	3 (27.3)	0.047
Fusidic acid	3 (8.1)	0 (0)	3 (27.3)	0.023
Phosphomycin	11 (29.7)	0 (0)	11 (100)	< 0.001

*n (%): number/percentage

Table 2 shows the resistance patterns for MRSA and MRCoNS. A total of 12 resistance patterns were obtained. Differences were found between patterns obtained for MRSA and MRCoNS, with 7 different patterns for MRSA and 5 for MRCoNS.

Discussion

Our study showed that goats in the Canary Islands are frequently colonised by MRSA-ST398. The presence of these multiresistant bacteria had been previously studied in pigs and livestock workers on the island of Tenerife but they had not been isolated in goats (Morcillo et al., 2012). The presence of livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) CC398 could indicate that these multiresistant strains have passed, in this habitat, from pigs to goats, due to some livestock contact in farms or even through human carriers. LA-MRSA CC398 strains have been described in many countries in Europe and worldwide as being prevalent in pigs and workers of the sector (Morcil-

lo et al., 2012; Chuang and Huang, 2015; Sahibzada et al., 2017). Accordingly, various authors stressed the importance of the occupational hazard caused by LA-MRSA CC398, with a higher colonisation in exposed workers than in the overall population (Goerge et al., 2017; Anker et al., 2018).

Table 2

Resistance patterns of the strains studied

Pattern	Total (%)	Microorganism	
		MRSA (%)	MRCoNS (%)
PG+OXA+STX	10 (27.8)	10 (40.0)	0 (0)
PG+OXA	6 (16.7)	6 (24)	0 (0)
PG+OXA+FM+E	4 (11.1)	0 (0)	4 (36.4)
PG+OXA+CC+E	3 (8.3)	3 (12.0)	0 (0)
PG+OXA+FM	3 (8.3)	0 (0)	3 (27.3)
PG+OXA+CC+STX	2 (5.6)	2 (8.0)	0 (0)
PG+OXA+CC+STX+FM+FA+E	2 (5.6)	0 (0)	2 (18.2)
PG + OXA + GM+TM+STX	2 (5.6)	2 (8.0)	0 (0)
PG+OXA+CC+STX+E+GM+TM	1 (2.8)	1 (4.0)	0 (0)
PG+OXA+CC+FM+E	1 (2.8)	0 (0)	1 (9.1)
PG+OXA+CC+FM+E+FA	1 (2.8)	0 (0)	1 (9.1)
PG+OXA+STX+E	1 (2.8)	1 (4.0)	0 (0)

*PG: benzylpenicillin, OXA: oxacillin, GM: gentamicin, TM: tobramycin, CC: clindamycin, E: erythromycin, SXT: trimethoprim-sulphamethoxazole, FA: fusidic acid, FM: phosphomycin

Several studies have reported the detection of MRSA strains in goat milk (Traversa et al., 2015; Parisi et al., 2016; Obaidat et al., 2018), considering it to be a major finding, as such strains had not been detected before in this type of livestock. Cortimiglia et al. (2015) demonstrated that the same MRSA strain was able to persist over time on the farm, being isolated from both the bulk tank milk and the udder of three goats. However, Porrero et al. (2012) did not find the *mecA* gene to be present in *S. aureus* from goat and sheep mastitis samples in Spain.

There are very few studies on MRSA colonisation in healthy goats. Alves et al. (2009) indicated that, contrary to the high presence of MRSA in other animals such as pigs and horses, its prevalence in ruminants was very low. Aquino et al. (2012) analysed 23 nasal samples from goats and did not find methicillin-resistant staphylococci; however, they found them in the staff of a teaching and research farm. Loncaric et al. (2013) reported, for the first time in Austria, the infection of a goat by the MRSA ST 398 strain, as well as the colonisation of other goats from the same herd and its suspected transmission to humans, resulting in colonisation in a farmer in charge of feeding them. The study indicates that none of the animals or humans were on antibiotic treatment, nor did they show any clinical signs.

We consider it important that our study has revealed the presence of strains with the *mecA* gene in different *Staphylococcus* species, as they are an important reservoir of resistance genes amounting to a serious public and animal health concern (Zhang et al., 2009; Huber et al., 2011; Nemeghaire et al., 2014).

The chromogenic medium used for the screening of MRSA (ChromID® MRSA, (BioMérieux® SA) allowed us to detect the presence of other methicillin-resistant coagulase-negative *Staphylococcus* bacteria. Other authors have used the same medium to detect MRCoNS strains (Nemeghaire et al., 2014). We thus consider that it could be an appropriate medium for screening methicillin-resistant coagulase-negative staphylococci growing as white colonies.

Several studies show the presence of MRCoNS strains in various animals and animal-derived products, such as meat and milk (Bhargava and Zhang, 2014; Nemeghaire et al., 2014), and the importance of these strains in workers in contact with the animals (Huber et al., 2011). In our study, we have identified 6 different species colonising goat nostrils, *S. lentus* and *S. sciuri* being predominant. In a study carried out in the United States, Zhang et al. (2009) found the presence of MRCoNS strains in different farm animals, including 10 goats, identifying the same species that we did.

The strains in our study present high resistance to other non-beta-lactam antibiotics. MRSA strains were resistant, in a high percentage, to co-trimoxazole (75%), minor in the case of MRCoNS, with significant differences. The main resistance pattern was multiresistance to non-carbapenem beta-lactams, together with co-trimoxazole which appears in 40% of the strains studied. Another important aspect to consider is resistance to erythromycin, clindamycin and to the aminoglycosides gentamicin and tobramycin.

MRCoNS strains present high resistance, especially to phosphomycin (100% of the strains studied) and erythromycin (72.7%), resistance to beta-lactams being the main pattern together with these two antimicrobials (36.4%).

In 2014 Spain was the biggest user of veterinary antimicrobials among the countries that submitted data to the European Surveillance Project of Veterinary Antimicrobial Consumption and, according to the Spanish Agency of Medicines and Healthcare Products, sales continued to increase in 2015 (European Commission, 2016). We consider these resistance levels to be very high and, in our opinion, this could be related to the significant consumption of existing antibiotics in livestock in Spain.

It was a limitation of our study that we were authorised to perform only nasal screening. More studies, such as rectal colonisation screening, are needed for the surveillance of multidrug-resistant bacteria.

This study is the first to report MRSA ST398 and coagulase-negative *Staphylococcu*s strains in goats in Spain, with a high resistance to also other groups of antibiotics. In conclusion, the high prevalence of MRSA and MRCoNS in the goats studied indicates the need to further study this strain of animal origin, as well as to increase surveillance and control measures at all stages of the food chain.

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