Prevalence, phenotypic and genotypic characters of *Staphylococcus aureus* isolated from mastitis in small ruminants in Italy

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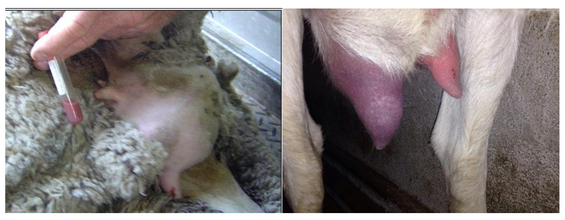
Abstract

Acute and chronic staphylococcal mastitis is a common disease of small ruminants causing major economic losses. The problem is even more relevant in the rural areas of the Mediterranean region, where almost two thirds of the global sheep and a quarter of the global goat milk are produced. Mastitis cause massive financial losses due to the reduced milk yield, price reductions with reduced milk quality and significantly increased premature loss of animals.

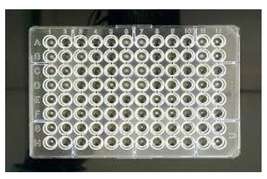
The aim of the study was to compare the genotypes, antimicrobial resistance (AMR) profiles and virulence factors of *S. aureus* strains from clinical mastitis in small ruminants farms in Sardinia, Italy. Whole-genome sequencing (WGS) of strains was conducted and the sequence data were analyzed regarding AMR and virulence genes to draw a conclusion for a current situation of small ruminants' clinical mastitis infections in dairy herds in the state and the potential public health risk. Furthermore, mapping the possible phylogenetic relations between *S. aureus* strains from various farms and within one farm was analyzed.

Isolation and Identification of *S. aureus* from mastitis cases

- Thirty four *S. aureus* isolates from milk were collected from sheep and goat with clinical mastitis from October 2021 to May 2022 from different 26 farms in 6 different provinces in Sardinia, Italy. The samples were cultured and tested according to the guidelines of the German Veterinary Association.
- All isolates were cultivated on Chromogenic agar medium.
- All isolates were confirmed as *S.aureus* using MALDI-TOF MS.
- WGS and antibiotic resistance testing were applied for all isolates.



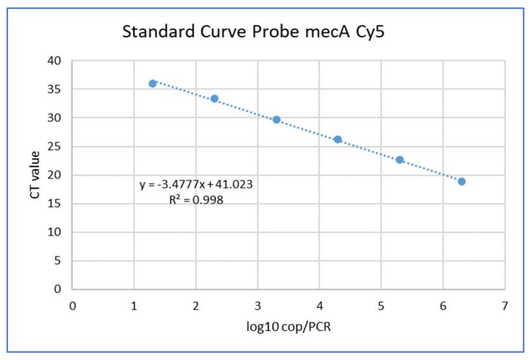
Phenotypic Antibiotic Resistance Profiles

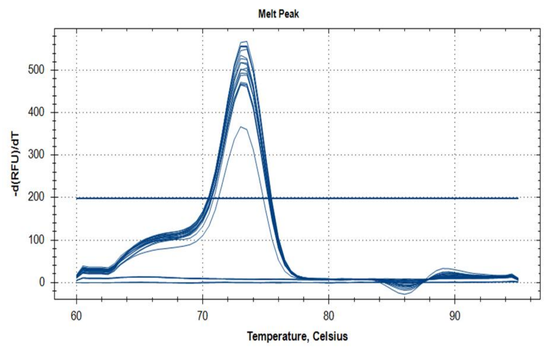


Antimicrobial class	Antimicrobial agent	Resistance	
		n.	%
Beta-lactams	penicillin	0	0
	Oxacillin	0	0
Aminoglycosides	Gentamicin	0	0
Fluoroquinolones	Ciprofloxacin	0	0
	Moxifloxacin	0	0
Macrolides	Erythromycin	1	2.9
Lincomycin	Clindamycin	0	0
Oxazolidinones	Linezolid	0	0
Glycopeptides	Teicoplanin	0	0
	Vancomycin	0	0
Tetracyclines	Tetracycline	5	14.7
	Tigecycline	0	0
Phosphonic antibiotics	Fosfomycin	0	0
Fusidane class	Fusidic acid	0	0
	Rifampicin	0	0
Sulfonamides plus trimethoprim	Trimethoprim/Sulfamethoxazole	0	0

Genetic characterization of *S. aureus*

Standard curve for *mec*A gene

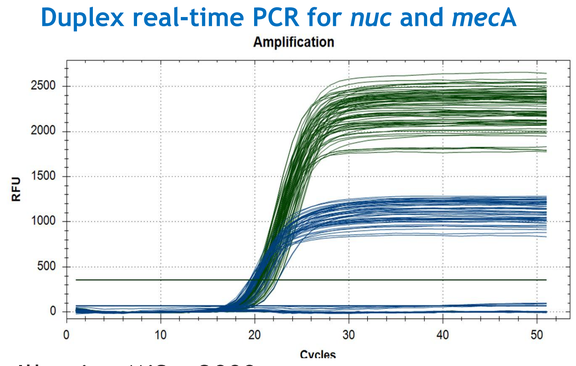




Primers used for confirmation of MRSA

Target gene	Primer, Probe	Nucleotide sequence (5'-3')	Target
nuc	nuc263-F	AAA GCG ATT GAT GGT GAT ACG GTT	*S. aureus*
	nuc355-R	TGC TTT GTT TCA GGT GTA TCA ACC A	
	nuc294-P	HEX- ATG TAC AAA GGT CAA CCA ATG ACA TTY AGA - BHQ1	
*mec*A	mecA-1501-F	GCT CAA ATT TCA AAC AAA AAT TTA GAT AAT G	MRSA
	mecA-1598-R	TGA AAG GAT CTG TAC TGG GTT AAT CAG T	
	mecA-1542-P	FAM- AGC TGA TTC AGG TTA CGG ACA AGG TGA- BHQ1	

Duplex real-time PCR for *nuc* and *mec*A

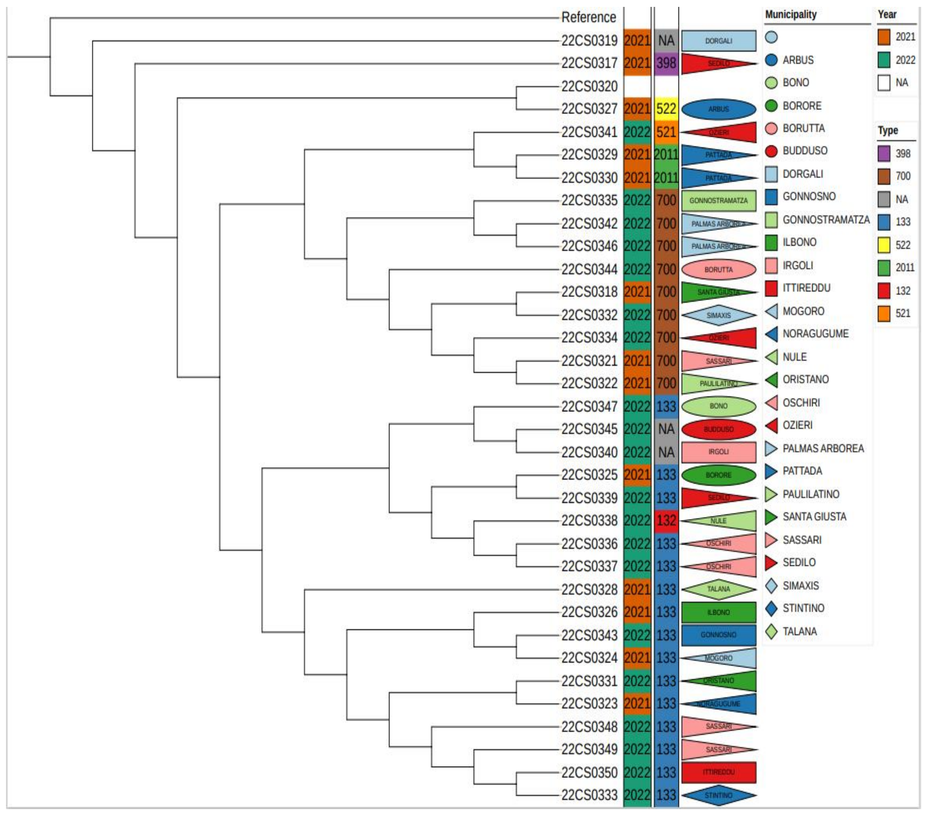


The extracted DNA was sequenced using an Illumina MiSeq2000 platform. Sequencing libraries were created using the Nextera XT DNA Library Preparation Kit (Illumina Inc., San Diego, CA). Paired end sequencing producing 300 bp long reads was performed on an Illumina MiSeq instrument according to the manufacturer's instructions (Illumina Inc., San Diego, CA). Raw sequencing data were deposited at the European Nucleotide Archive (ENA) as BioProject PRJEB61659. The Linux based bioinformatics pipeline WGSBAC v. 2.2.01 was used for data analysis.

Results

- All isolates proved to be phenotypically methicillin sensitive *S. aureus* (MSSA).
- Only few isolates showed resistance against tetracycline (14.7%) and erythromycin (2.9%).
- The isolates were assigned to seven different sequence types: ST133 (n = 15), ST700 (n = 9) were the main two sequence types.
- Resistance genes *bla*Z and *dfr*G were found in one isolate, each (2.9%).
- All tetracycline resistant isolates harbored either *tet*M or *tet*K.

Phylogenetic analysis tree of 34 *S. aureus* isolated from clinical mastitis cases from 26 farms in Sardinia using WGS analysis



The tree was rooted to the reference genome and visualized using the interactive Tree of Life (iTOL) v. 4 web tool. Data included are; Survey year, ST types, Municipality and Province

Conclusions

- Seven different sequence types (STs; 398, 700, 132, 133, 521, 522 and 2011) and associated clusters was found and geographically widely distributed among farms.
- All isolates were phenotypically sensitive to most of tested drugs except Erythromycin (2.9%) and Tetracycline (14.7%).
- ST133 had the highest potential to cause disease and was found frequently in sheep mastitis cases.
- Results of WGS were fully in accordance with phenotypic resistance of all isolates.
- Both of *tet*-M (2.9%) and *tet*-K (11.8%) genes were detected in isolates using WGS, both were conferring resistance to Tetracycline.
- Additionally Erythromycin associated resistance (*erm*) gene was identified in one isolate (2.9%) using WGS.
- The identification of CA-MRSA such as ST398 among isolates poses a high risk for zoonotic transmission and interplay of such strains between animals and humans on the farms.
- All isolates were obtained from clinical mastitis cases. However, the lowered rates of resistance among isolates indicate the highly developed hygienic measures in the surveyed farms, and the routine AMR testing in combination with prudent use of antimicrobials to avoid the emergence of resistance.
- This is in line with the current agricultural regulations, i.e., implementation of EU legislation, increases the farmers´ income through avoiding losses due to reduced milk yield and decreases the veterinary costs. Most importantly, the burden of disease is reduced and animal welfare is improved.

Acknowledgement

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