

Antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* from small ruminants in Portugal: detection of *mecA* and *mecC* in sheep

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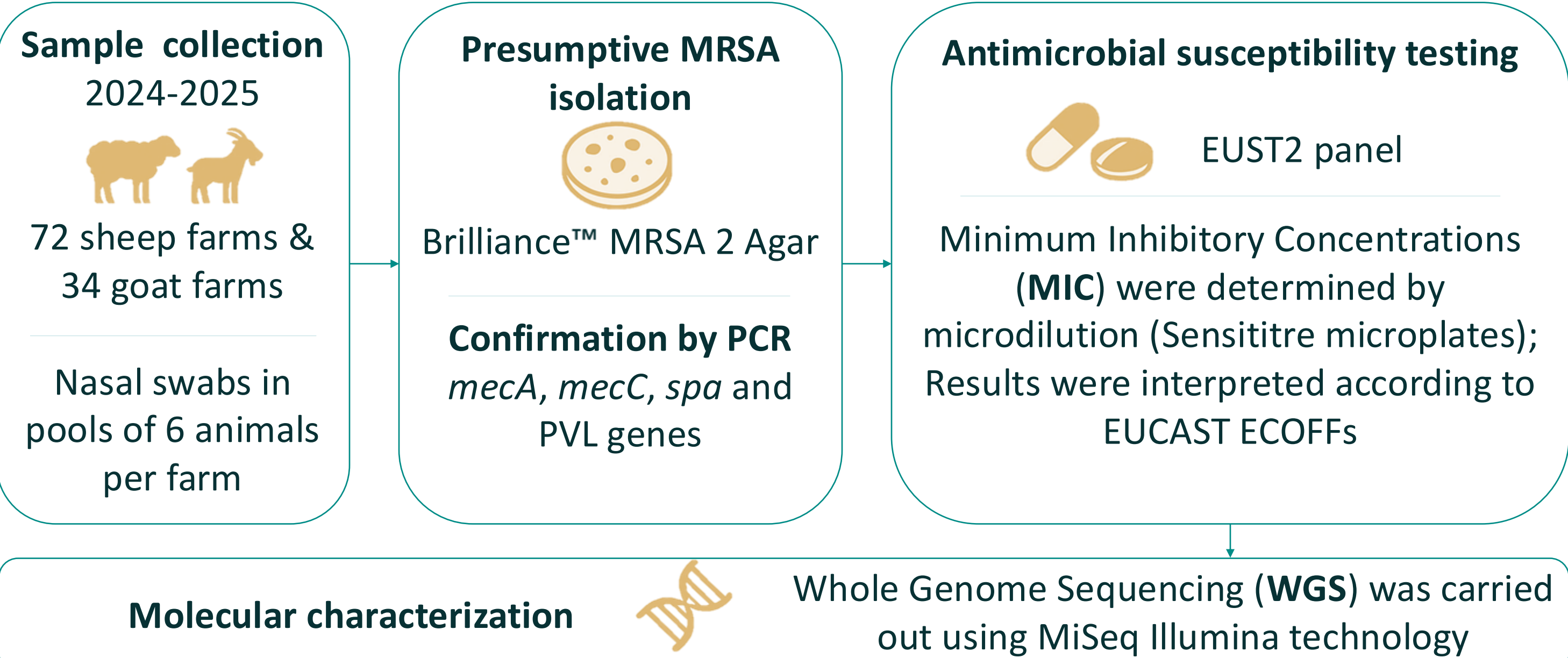
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BACKGROUND

Staphylococcus aureus is a natural colonizer of the nasal mucosa, and is considered an opportunistic pathogen (1). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant public health concern, but antimicrobial resistance data in small ruminants are scarce (1).

AIM OF THIS STUDY: To phenotypically and genotypically characterize MRSA isolates from sheep and goats (2024–2025) within a pilot antimicrobial resistance surveillance project.

METHODS



RESULTS

Presumptive MRSA were detected in 5.6% (4/72) sheep farms and 11.8% (4/34) goat farms. MRSA was confirmed in three strains. The distribution of antibiotic resistance versus susceptibility of presumptive MRSA is represented in **Figure 1**. The resistance phenotypes and WGS results of the strains are presented in **Table 1**.

- ✓ All isolates were susceptible to erythromycin, fusidic acid, gentamicin, kanamycin, linezolid, mupirocin, streptomycin, rifampicin, sulfamethoxazole, quinupristin/dalfopristin, trimethoprim, and vancomycin.
- ✓ Two isolates of sheep and three isolates of goats were resistant to ceftiofur (MIC= 8 µg/mL) but not related to *mec* genes.
- ✓ *Mec* genes were found in three isolates from different farms: two sheep isolates were resistant to ceftiofur (MICs of 8 and 16 µg/mL), harboring *mecC* and *mecA*, respectively. One goat isolate was resistant to ceftiofur (MIC =16 µg/mL) harboring *mecC*.



Figure 1. Antibiotic resistance distribution of presumptive MRSA isolates in sheep (A) and goats (B). FOX-Ceftiofur; CHL-Chloranphenicol; PEN-Penicillin; TET-tetracycline; CIP-Ciprofloxacin; CLI-Clindamycin; TIA-Tiamulin; ERY-Erythromycin; FUS-Fusidic acid; GEN-gentamicin; KAN-Kanamycin; LZD-Linezolid; MUP-Mupirocin; RIF-Rifampicin; STR-Streptomycin; SMX-Sulfamethoxazole; SYN-Quinupristin/dalfopristin; TMP-Trimethoprim; VAN-Vancomycin.

Animal	Isolate	Resistance phenotype	Genes	Plasmid	Efflux pumps	MLST
Goat	24-18751	FOX, TET	-	rep24b	mgrA, mepR	2328
Goat	24-15960	FOX, TET	<i>tet(M)</i>	repUS43	mepR, mgrA	5
Goat	24-15950	FOX	<i>tet(M)</i>	repUS43	mepR, mgrA	6048
Goat	24-27673	FOX, TET, PEN	<i>mecC</i> , <i>blaZ</i>	rep5c, repUS46	mgrA, mepR,	130
Sheep	24-15466	FOX	-	-	mgrA	133
Sheep	24-15771	FOX, CHL, TET	<i>mecC</i> e <i>blaZ</i>	repUS46, rep5c	mgrA, mepR, LmrS	130
Sheep	24-18750	FOX, CHL, CIP, CLI, TET, TIA, PEN	<i>mecA</i> , <i>blaZ</i> , <i>vga(A)</i> , <i>tet(M)</i> , <i>tet(K)</i> , <i>fexA</i>	rep7a, rep7b, repUS43	mgrA, fexA, LmrS, arlR, norC, nora, tet(K),	398
Sheep	24-16735	FOX, PEN	-	-	mgrA	1640

Table 1. Resistance phenotype, genes, plasmid and efflux pumps of the presumptive MRSA isolates. The genes in yellow and blue are in the same contig as the replicase from the same color plasmid. FOX-Ceftiofur; TET-Tetracycline; PEN-Penicillin; CHL-Chloranphenicol; CIP-Ciprofloxacin; CLI-Clindamycin; TIA-Tiamulin; MLST-Multi-Locus Sequence Typing.

WGS results are summarized in **Table 1**, showing the resistance genes, plasmids, and efflux pumps found in each isolate, along with the respective MLST.

- ✓ All MRSA are multidrug-resistant.
- ✓ The strain 24-18750 harbouring *mecA* belongs to the CC398 and carries the highest number of antibiotic resistance and efflux pumps genes on three plasmids.
- ✓ The *mec* genes were not linked to the replicase of any plasmids.
- ✓ Tet(M) resistance gene were found on the same contig as the replicase of the repUS43 plasmid, and vtga(A) gene of the rep7b plasmid.

The reference strain 1-44893 was used to construct the phylogenetic tree (**Figure 2**). For comparative purposes, three pig MRSA (INIAV001-INIAV003) of the CC398 isolated in our lab (2) were also included in the analysis. The *mecC* strains have only one SNP difference between them.

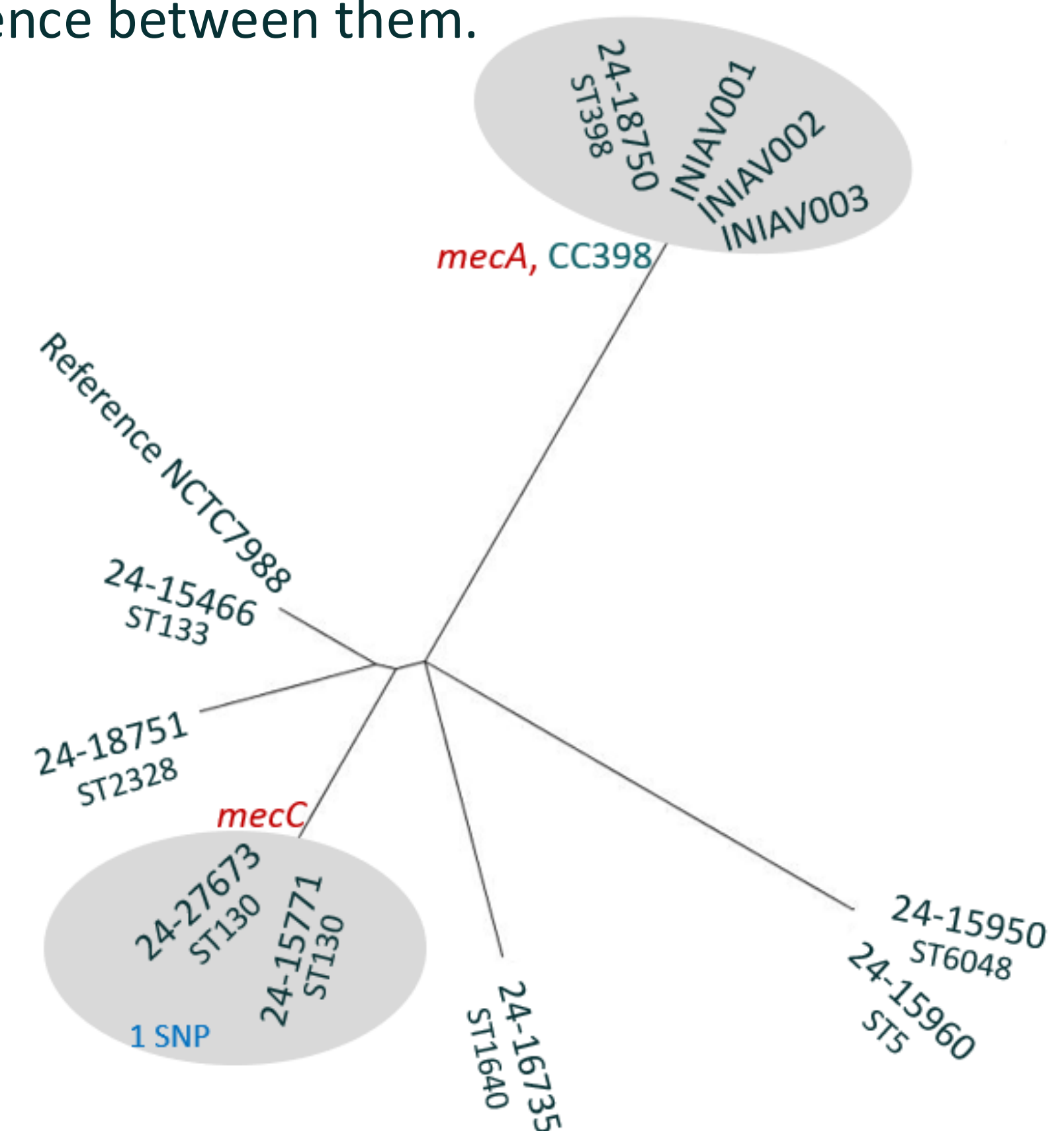


Figure 2. Phylogenetic tree of the eight strains of presumptive MRSA from sheep and goats. SNP-Single nucleotide polymorphisms.

CONCLUSIONS

- ✓ To our knowledge, this is the first report of *mecC* and *mecA* MRSA in small ruminants from Portugal.
- ✓ Five strains had low resistance to ceftiofur without harboring *mec* genes, suggesting other resistance mechanisms, likely mutations related to cell-wall metabolism and/or efflux pumps.
- ✓ Further studies on ceftiofur resistance not linked to *mec* genes are needed, given the implications for diagnosis and treatment.
- ✓ This study highlights the importance of surveillance in small ruminants for understanding the current epidemiological situation and promoting responsible antimicrobial use.

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