

Antimicrobial susceptibility and molecular typing of *Streptococcus uberis* isolates collected from sheep milk samples

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Introduction

Mastitis is the one of the most common health problems affecting dairy sheep. Sardinia, an island located in the middle of the Mediterranean Sea, has approximately 3.2 million milking Sarda sheep, corresponding to half of the total Italian stock. A considerable part of the regional economy is based on dairy sheep farming, mainly for pecorino cheese production. Therefore, the control of intra-mammary infections (IMI) is of greatest importance for dairy farmers. IMI outbreaks are usually caused by Gram-positive bacteria, mostly *Staphylococcus* and *Streptococcus* species [1]. *Streptococcus uberis* is considered an environmental pathogen since it is commonly isolated from the environment, milking machines, milkers' hands, and the skin of the teats. Mastitis may therefore arise when the mammary gland is exposed to high loads of this bacterial species.

Materials and Methods

A bank of 124 *S. uberis*, collected from sheep milk samples in different provinces of Sardinia (Italy), were analysed. Bacterial identification at the species level was performed by PCR-RFLP and MALDI-TOF MS analysis [2]. All isolates were screened for resistance to 14 antimicrobial agents according to CLSI recommendations (Fig. 1). Amplification of the seven housekeeping genes (*arcC*, *ddl*, *gki*, *recP*, *tdk*, *tpi* and *yqiL*) was performed using the primer sequences as described by Coffey et al [3]. The allelic profile of each isolate was identified after sequencing and, where possible, strains were assigned to sequence type (ST) using the *S. uberis* MLST database (<http://pubmlst.org/organisms/streptococcus-uberis>).

Results and Discussion

Almost all *S. uberis* isolates (120/124, 96.8%) were resistant to at least one antimicrobial. Resistance was exhibited to all the antimicrobials tested, except for amoxicillin-clavulanic acid, oxacillin, and ceftiofur. Of the antimicrobials tested, the highest resistance rate was recorded against streptomycin (116/124, 93.5%), kanamycin (99/124, 79.8%) and gentamicin (80/124, 64.5%), followed by novobiocin (31/124, 25%), and tetracycline (24/124, 19.3%). Seventy-four (59.7%) isolates were simultaneously resistant to all aminoglycosides. Seventeen isolates (13.7%) were MDR: ten were resistant to three different classes, six to four different classes and one to five different classes (penicillin, tetracycline, streptomycin, trimethoprim-sulphamethoxazole and novobiocin)(Fig. 2). MLST analysis identified 86 different allelic combinations; among these, 13 were STs present in the database while the remaining 73 (84.9%) STs were found for the first time (Fig 3). CC143 was the most abundant clonal complex among the ovine *S. uberis* isolates, followed by CC86 and CC5. Genotyping by MLST analysis indicates the epidemiological complexity of *S. uberis* isolated from ovine milk.

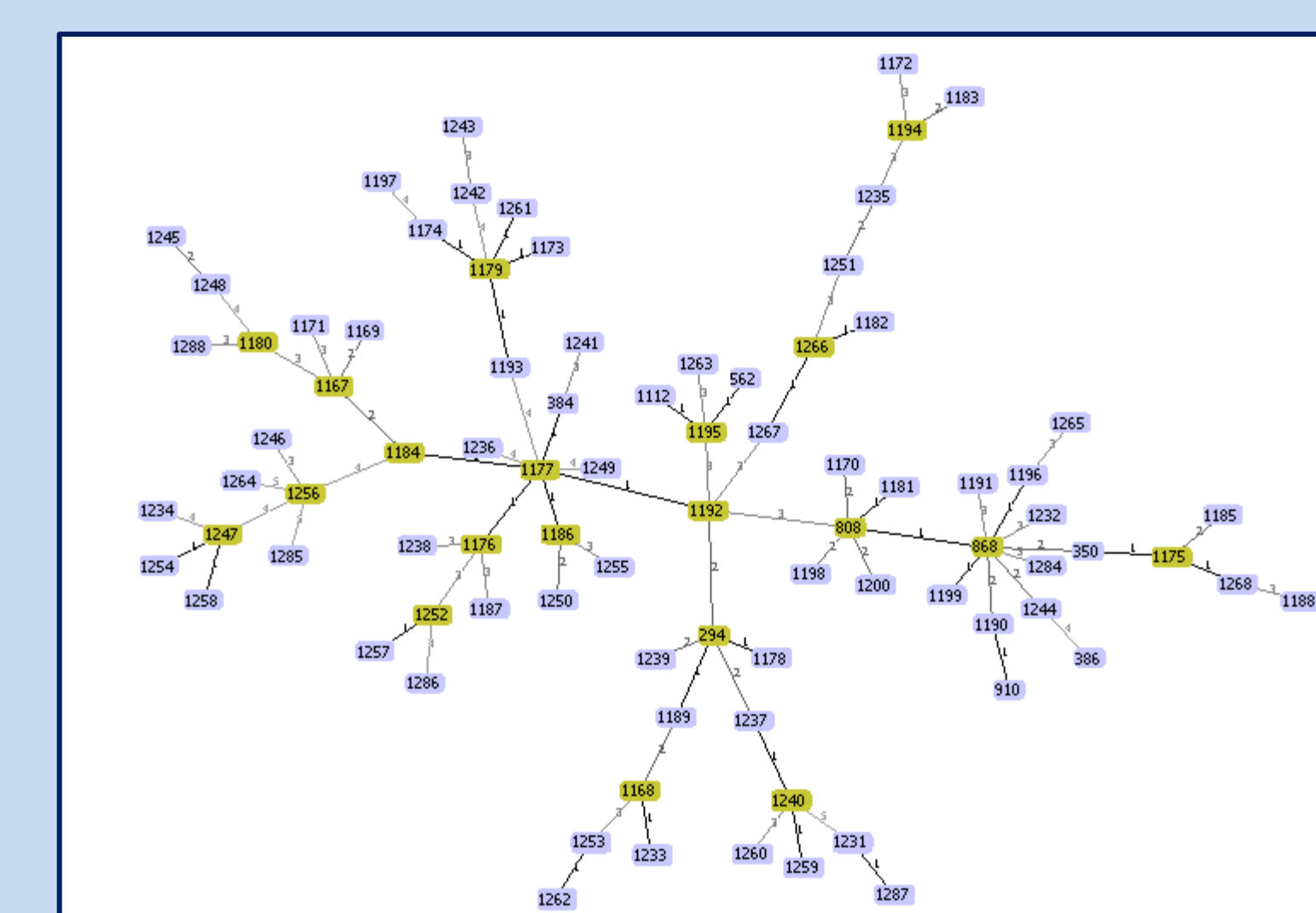


Fig. 3. A complete minimum spanning tree (MST) based on allelic profiles of *Streptococcus uberis* isolated from ovine milk samples in Sardinia, Italy. The tree was generated by goeBURST full MST algorithm in PHYLOVIZ 2.0 (<http://www.phyloviz.net>) (accessed on 29 April 2021). Numbers within the nodes indicate the ST. Yellow nodes correspond to the possible ST founders. Numbers on lines indicate locus variants between adjacent nodes.



References

- [1] Marogna G, Rolesu S, Lollai S, Tola S, et al. 2010. Small Rumin. Res. 88:119-125. [2] Rosa MN, Agnoletti F, Lollai S, Tola S. 2019. Small Rumin. Res. 180:35-40. [3] Coffey TJ, Pullinger GD, Urwin R, Jolley KA, et al. 2006. Appl. Environ. Microbiol. 72:1420-1428.