

# MLST-Based Analysis and Resistance to Antimicrobial Agents of *Staphylococcus epidermidis* from Cases of Sheep Mastitis in Greece

Eleni I. Katsarou, Dimitrios C. Chatzopoulos, Themis Giannoulis, Katerina S. Ioannidi, Angeliki I. Katsafadou, Panagiota I. Kontou, Daphne S. Lianou, Zissis Mamuris, Vasia S. Mavrogianni, Charalambia K. Michael, Elias Papadopoulos, Efthimia Petinaki, Styliani Sarrou, Natalia G.C. Vasileiou, George C. Fthenakis

**Table S1.** Primers used for detection of genes for inclusion in the MLST database, in 33 *S. epidermidis* isolates recovered from subclinical mastitis in ewes in Greece (MLST: Multi Locus Sequence Typing).

| Gene                                               | Primers | Sequences                  | Product size (bp) |
|----------------------------------------------------|---------|----------------------------|-------------------|
| <i>arcC</i> (carbamate kinase)                     | arcC-F  | TGTGATGAGCACGCTACCGTTAG    | 465               |
|                                                    | arcC-R  | TCCAAGTAAACCCATCGGTCTG     |                   |
| <i>aroE</i> (shikimate dehydrogenase)              | aroE-F  | CATTGGATTACCTCTTTGTTTCAGC  | 420               |
|                                                    | aroE-R  | CAAGCGAAATCTGTTGGGG        |                   |
| <i>gtr</i> (ABC transporter)                       | gtr-F   | CAGCCAATTCTTTTATGACTTTT    | 438               |
|                                                    | gtr-R   | GTGATTAAAGGTATTGATTGAAT    |                   |
| <i>mutS</i> (DNA mismatch repair protein)          | mutS-F3 | GATATAAGAATAAGGGTTGTGAA    | 412               |
|                                                    | mutS-R3 | GTAATCGTCTCAGTTATCATGTT    |                   |
| <i>pyrR</i> (pyrimidine operon regulatory protein) | pyr-F2  | GTTACTAATACTTTTGCTGTGTTT   | 428               |
|                                                    | pyr-R4  | GTAGAATGTAAAGAGACTAAAATGAA |                   |
| <i>tpiA</i> (triosephosphate isomerase)            | tpi-F2  | ATCCAATTAGACGCTTTAGTAAC    | 424               |
|                                                    | tpi-R2  | TTAATGATGCGCCACCTACA       |                   |
| <i>yqiL</i> (acetyl coenzyme A acetyltransferase)  | yqiL-F2 | CACGCATAGTATTAGCTGAAG      | 416               |
|                                                    | yqiL-R2 | CTAATGCCTTCATCTTGAGAAATAA  |                   |

**Table S2.** Primers used and work conditions undertaken for detection of resistance genes in 33 *S. epidermidis* isolates recovered from subclinical mastitis in ewes in Greece.

| Gene        | Primer sequence                                                      | Concentration ( $\mu$ M) | Product size (bp) | Annealing temperature ( $^{\circ}$ C) | Reference |
|-------------|----------------------------------------------------------------------|--------------------------|-------------------|---------------------------------------|-----------|
| <i>ermA</i> | Fw-TCTAAAAAGCATGTAAAAGAA<br>Rv-CTTCGATAGTTTATTAATATTAGT              | 0.2                      | 645               | 55                                    | [1]       |
| <i>ermB</i> | Fw-GAAAAGGATCTCAACCAAATA<br>Rv-AGTAACGGTACTTAAATTGTTTAC              | 0.2                      | 639               | 55                                    | [1]       |
| <i>ermC</i> | Fw-TCAAAACATAATATAGATAAA<br>Rv-GCTAATATTGTTTAAATCGTCAAT              | 0.2                      | 649               | 52                                    | [1]       |
| <i>ermT</i> | Fw-GGTGTAATTATGTAACCGCCA<br>Rv-ACTTCCTGTAGCTGTGCTTTC                 | 0.2                      | 218               | 52                                    | [1]       |
| <i>ermY</i> | Fw-ATTACTTCGAAACGTAATATAGAT<br>Rv-ATAGCTATTGAAAAGAGACAAGA            | 0.2                      | ~ 700             | 60                                    | [2]       |
| <i>lnuA</i> | Fw-GGTGGCTGGGGGGTAGATGTATTAAGTGG<br>Rv-GCTTCTTTTGAAATACATGGTATTTTCGA | 0.2                      | 323               | 55                                    | [3]       |
| <i>lnuB</i> | Fw-CCTACCTATTGTTTGTGGAA<br>Rv-ATAACGTTACTCTCCTATTC                   | 0.2                      | 944               | 50                                    | [4]       |
| <i>lnuC</i> | Fw-GTAGATGCTCTTCTTGGAT<br>Rv-TTCTACCGGAAAACAATCC                     | 0.2                      | 246               | 55                                    | [5]       |
| <i>lsaA</i> | Fw-GATCAGGCAAATATCACGATG<br>Rv-CTTCATGAAATGTGCATCATGC                | 0.2                      | ~ 1400            | 60                                    | [6]       |
| <i>mphC</i> | Fw-ATCTCATTGAATGAATCAGGAC<br>Rv-CTACTCTTCCATACCTAACTC                | 0.2                      | ~ 850             | 60                                    | [2]       |
| <i>msrA</i> | Fw-GCAAATGGTGTAGGTAAGACAACCT<br>Rv- ATCATGTGATGTAAACAAAAT            | 0.2                      | 399               | 55                                    | [1]       |
| <i>tetK</i> | Fw-TATTTTGGCTTTGTATTCTTTCAT<br>Rv-GCTATACCTGTTCCCTCTGATAA            | 0.2                      | 1159              | 59                                    | [7]       |
| <i>tetL</i> | Fw-ATAAATTGTTTCGGGTCGGTAAT<br>Rv-AACCAGCCAACTAATGACAATG              | 0.2                      | 1077              | 59                                    | [7]       |
| <i>tetM</i> | Fw-GAACTCGAACAAGAGGAAAGC<br>Rv-ATGGAAGCCCAGAAAGGAT                   | 0.2                      | 740               | 54                                    | [8]       |
| <i>tetS</i> | Fw-ATCAAGATATTAAGGAC<br>Rv-TTCTCTATGTGGTAATC                         | 0.2                      | 573               | 55                                    | [9]       |

|             |                                                       |     |     |    |      |
|-------------|-------------------------------------------------------|-----|-----|----|------|
| <i>tetT</i> | Fw-CAGTGCGAATATAAGGACACGTC<br>Rv-CAAGCCTTCTCTACAGCATC | 0.2 | 644 | 61 | [10] |
| <i>vgaA</i> | Fw-CTTGTCTCCTCCGCGAATAC<br>Rv-AGTGGTGGTGAAGTAACACG    | 0.2 | 659 | 55 | [11] |

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**Table S3.** Husbandry factors applied in sheep flocks, which were evaluated for potential association with resistance to antimicrobial agents of 33 *S. epidermidis* isolates recovered from subclinical mastitis in ewes in Greece.

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|                                                                                                  |
|--------------------------------------------------------------------------------------------------|
| Management system applied in the flock (description according to EFSA classification) [1]        |
| Stage of the lactation period at sampling (month)                                                |
| Milking technique applied in the flock (machine milking, hand-milking)                           |
| Application of post-milking teat dipping (yes, no)                                               |
| Intramammary administration of antimicrobial agents at the end of the lactation period (yes, no) |
| Vaccination against staphylococcal mastitis (yes, no)                                            |

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#### References

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**Table S4.** Identity and details in the MLST database of 33 *S. epidermidis* isolates recovered from subclinical mastitis in ewes in Greece (MLST: Multi Locus Sequence Typing).

| Isolate ID | Alleles of relevant genes |             |            |             |             |             |             | ST               |
|------------|---------------------------|-------------|------------|-------------|-------------|-------------|-------------|------------------|
|            | <i>arcC</i>               | <i>aroE</i> | <i>gtr</i> | <i>mutS</i> | <i>pyrR</i> | <i>tpiA</i> | <i>yqiL</i> |                  |
| 1134       | 2                         | 1           | 1          | 17          | 20          | 1           | 1           | 709              |
| 1135       | 8                         | 51          | 56         | 37          | 9           | 16          | 1           | 710              |
| 1136       | 1                         | 1           | 57         | 6           | 2           | 16          | 7           | 711              |
| 1143       | 65                        | 59          | 5          | 5           | 8           | 48          | 11          | 700              |
| 1316       | 12                        | 25          | 9          | 8           | 6           | 5           | 8           | 315              |
| 1317       | 1                         | 1           | 2          | 2           | 3           | 1           | 3           | 200              |
| 1318       | 2                         | 1           | 2          | 2           | 2           | 1           | 1           | 142              |
| 1319       | 2                         | 1           | 2          | 2           | 2           | 1           | 1           | 142              |
| 1320       | 2                         | 1           | 2          | 2           | 2           | 1           | 1           | 142              |
| 1321       | 1                         | 1           | 2          | 6           | 2           | 1           | 1           | 152              |
| 1323       | 1                         | 1           | 2          | 6           | 2           | 1           | 1           | 152              |
| 1325       | 1                         | 1           | 2          | 6           | 2           | 1           | 1           | 152              |
| 1327       | 1                         | 1           | 2          | 1           | 2           | 1           | 8           | 454 <sup>1</sup> |
| 1328       | 2                         | 1           | 1          | 2           | 20          | 1           | 1           | 100              |
| 1329       | 2                         | 1           | 1          | 2           | 20          | 1           | 1           | 100              |
| 1330       | 2                         | 1           | 1          | 2           | 20          | 1           | 1           | 100              |
| 1331       | 2                         | 1           | 1          | 1           | 2           | 1           | 1           | 59               |
| 1332       | 1                         | 1           | 2          | 6           | 2           | 16          | 7           | 570              |
| 1333       | 2                         | 1           | 2          | 2           | 2           | 2           | 1           | 142              |
| 1366       | 8                         | 2           | 1          | 1           | 2           | 1           | 1           | 677              |
| 1367       | 2                         | 1           | 2          | 2           | 2           | 1           | 1           | 142              |
| 1368       | 2                         | 1           | 1          | 2           | 20          | 1           | 1           | 100              |
| 1369       | 2                         | 1           | 1          | 17          | 20          | 1           | 1           | 709              |
| 1370       | 8                         | 51          | 56         | 37          | 9           | 16          | 1           | 710              |
| 1371       | 2                         | 1           | 1          | 2           | 20          | 1           | 1           | 100              |
| 1372       | 2                         | 1           | 1          | 2           | 20          | 1           | 1           | 100              |
| 1373       | 2                         | 1           | 1          | 2           | 20          | 1           | 1           | 100              |
| 1374       | 2                         | 1           | 6          | 2           | 2           | 2           | 1           | 153              |
| 1375       | 1                         | 1           | 2          | 6           | 2           | 1           | 1           | 152              |
| 1376       | 1                         | 1           | 2          | 6           | 2           | 1           | 1           | 152              |
| 1377       | 65                        | 59          | 5          | 5           | 8           | 48          | 11          | 700              |
| 1378       | 2                         | 1           | 2          | 2           | 2           | 1           | 7           | 678              |
| 1379       | 2                         | 1           | 2          | 2           | 2           | 1           | 7           | 678              |

<sup>1</sup> Red lettering indicates missing alleles (completed by means of an imputational method) and missing STs (thereafter completed as the most probable ST).

**Table S5.** Profiles of resistance to antimicrobial agents of 33 *S. epidermidis* isolates recovered from subclinical mastitis in ewes in Greece.

| Isolate identity in the MLST database <sup>1</sup> | Antimicrobial agents to which resistance was recorded <sup>2</sup> |
|----------------------------------------------------|--------------------------------------------------------------------|
| 1134                                               | P, AMP                                                             |
| 1135                                               | TE, FA                                                             |
| 1136                                               | none                                                               |
| 1143                                               | E, TE                                                              |
| 1316                                               | none                                                               |
| 1317                                               | P, AMP, TE                                                         |
| 1318                                               | P, AMP, FA                                                         |
| 1319                                               | P, AMP, TE                                                         |
| 1320                                               | P, AMP, TE                                                         |
| 1321                                               | P, AMP, TE                                                         |
| 1323                                               | P, AMP, TE                                                         |
| 1325                                               | none                                                               |
| 1327                                               | none                                                               |
| 1328                                               | none                                                               |
| 1329                                               | none                                                               |
| 1330                                               | none                                                               |
| 1331                                               | E, CC,TE, FOX                                                      |
| 1332                                               | none                                                               |
| 1333                                               | none                                                               |
| 1366                                               | P, AMP, TE, FA                                                     |
| 1367                                               | P, AMP, TE                                                         |
| 1368                                               | P, AMP, TE                                                         |
| 1369                                               | P, AMP                                                             |
| 1370                                               | TE, FA                                                             |
| 1371                                               | TE                                                                 |
| 1372                                               | none                                                               |
| 1373                                               | TE                                                                 |
| 1374                                               | none                                                               |
| 1375                                               | none                                                               |
| 1376                                               | none                                                               |
| 1377                                               | E, TE                                                              |
| 1378                                               | TE                                                                 |
| 1379                                               | none                                                               |

<sup>1</sup> MLST: Multi Locus Sequence Typing, <sup>2</sup> AMP: ampicillin, CC: clindamycin, E: erythromycin, FA: fucidic acid, FOX: ceftiofur, P: penicillin, TE: tetracycline.

**Table S6.** Frequency of *S. epidermidis* isolates from cattle farms and of isolates from sheep flocks, machine-or hand-milked, in Greece, according to STs in the MLST database (MLST: Multi Locus Sequence Typing, ST: sequence type).

| Isolates from cattle farms <sup>1</sup> |                        | Isolates from sheep flocks with machine-milking |                        | Isolates from sheep flocks with hand-milking |                        |
|-----------------------------------------|------------------------|-------------------------------------------------|------------------------|----------------------------------------------|------------------------|
| ST (n=36)                               | no. of isolates (n=50) | ST (n=8)                                        | no. of isolates (n=12) | ST (n=10)                                    | no. of isolates (n=21) |
| 5                                       | 2                      | 59                                              | 1                      | 100                                          | 5                      |
| 7                                       | 1                      | 100                                             | 2                      | 142                                          | 2                      |
| 48                                      | 2                      | 142                                             | 3                      | 152                                          | 3                      |
| 54                                      | 1                      | 152                                             | 2                      | 153                                          | 1                      |
| 57                                      | 1                      | 200                                             | 1                      | 454                                          | 1                      |
| 59                                      | 5                      | 315                                             | 1                      | 677                                          | 1                      |
| 91                                      | 6                      | 570                                             | 1                      | 678                                          | 2                      |
| 92                                      | 1                      | 711                                             | 1                      | 700                                          | 2                      |
| 93                                      | 1                      |                                                 |                        | 709                                          | 2                      |
| 94                                      | 2                      |                                                 |                        | 710                                          | 2                      |
| 98                                      | 1                      |                                                 |                        |                                              |                        |
| 99                                      | 1                      |                                                 |                        |                                              |                        |
| 100                                     | 1                      |                                                 |                        |                                              |                        |
| 101                                     | 1                      |                                                 |                        |                                              |                        |
| 102                                     | 1                      |                                                 |                        |                                              |                        |
| 103                                     | 1                      |                                                 |                        |                                              |                        |
| 104                                     | 1                      |                                                 |                        |                                              |                        |
| 105                                     | 1                      |                                                 |                        |                                              |                        |
| 106                                     | 1                      |                                                 |                        |                                              |                        |
| 107                                     | 1                      |                                                 |                        |                                              |                        |
| 108                                     | 1                      |                                                 |                        |                                              |                        |
| 109                                     | 2                      |                                                 |                        |                                              |                        |
| 110                                     | 1                      |                                                 |                        |                                              |                        |
| 111                                     | 2                      |                                                 |                        |                                              |                        |
| 112                                     | 1                      |                                                 |                        |                                              |                        |
| 114                                     | 1                      |                                                 |                        |                                              |                        |
| 329                                     | 1                      |                                                 |                        |                                              |                        |
| 441                                     | 1                      |                                                 |                        |                                              |                        |
| 443                                     | 1                      |                                                 |                        |                                              |                        |
| 444                                     | 1                      |                                                 |                        |                                              |                        |
| 452                                     | 1                      |                                                 |                        |                                              |                        |
| 453                                     | 1                      |                                                 |                        |                                              |                        |
| 454                                     | 1                      |                                                 |                        |                                              |                        |
| 473                                     | 1                      |                                                 |                        |                                              |                        |
| 474                                     | 1                      |                                                 |                        |                                              |                        |
| 639                                     | 1                      |                                                 |                        |                                              |                        |

1. Details obtained from the MLST-database.

**Table S7.** STs with *S. epidermidis* isolates from sheep flocks, machine-or hand-milked, in Greece, with simultaneous inclusion of isolates from human sources in the MLST database (MLST: Multi Locus Sequence Typing, ST: sequence type).

| STs with isolates from sheep flocks<br>with machine-milking (n=8) | STs with isolates from sheep flocks<br>with hand-milking (n=10) | Inclusion of isolates from<br>human sources in the ST |
|-------------------------------------------------------------------|-----------------------------------------------------------------|-------------------------------------------------------|
| 59                                                                |                                                                 | yes                                                   |
| 100                                                               | 100                                                             | no                                                    |
| 142                                                               | 142                                                             | yes                                                   |
| 152                                                               | 152                                                             | yes                                                   |
|                                                                   | 153                                                             | yes                                                   |
| 200                                                               |                                                                 | yes                                                   |
| 315                                                               |                                                                 | yes                                                   |
|                                                                   | 454                                                             | no                                                    |
| 570                                                               |                                                                 | no                                                    |
|                                                                   | 677                                                             | no                                                    |
|                                                                   | 678                                                             | no                                                    |
|                                                                   | 700                                                             | no                                                    |
|                                                                   | 709                                                             | no                                                    |
|                                                                   | 710                                                             | no                                                    |
| 711                                                               |                                                                 | no                                                    |



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