**Characterization of penicillin and tetracycline resistance in *Staphylococcus aureus* isolated from bovine milk samples in Minas Gerais, Brazil**

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## Supplementary File

Additional information about the methods:

*Minimum inhibitory concentrations*
*S. aureus* ATCC 29213 was tested in parallel in each batch of the isolates as a test quality control. The breakpoint values for resistance to ampicillin, penicillin and tetracycline were ≥ 0.5 µg/mL, ≥ 0.25 µg/mL and ≥ 16 µg/mL, respectively, according to CLSI (2013). The results of MIC were reported considering two-fold dilutions centred at 1 µg/mL. The half-log MIC values were interpreted to the next higher value on the standard two-fold dilution series.

*Beta-lactamase production*
*S. aureus* ATCC 29213 was used as a positive control for beta-lactamase production, and *S. aureus* ATCC 25923 was used as a negative control (CLSI 2013). The negative isolates were further evaluated for the induction of beta-lactamase by testing the colony material from the edge of the inhibition zone around the oxacillin disk (1 µg, Oxoid, Hampshire, England) in Mueller-Hinton Agar (BD, Sparks, USA) according to CLSI (2013).

*Amplification of the resistance genes*
Bacterial strains from a collection of bovine mastitis isolates from Embrapa Dairy Cattle Research Center were used as positive controls for the amplification of the resistance genes. The PCR reactions were performed on a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Carlsbad, EUA). The amplified DNA fragments were visualized following agarose gel electrophoresis (1%, w/v) and stained with an ethidium bromide solution (0.005% w/v). The images were recorded in a gel imaging system (Eagle Eye II, Stratagene, La Jolla, USA).

*Sequence analysis of the amplicons*
Amplicons were purified from the PCR mixture using the Easy Gen kit (Easy Path, São Paulo, Brazil). The primers used for the sequencing reactions were the same as those used for the PCR amplifications. Both the forward and reverse sequencing reactions were performed with two sequencing reads per sample.

Supplementary Table S1. The origin of the 90 *Staphylococcus aureus* isolates from bovine mastitis investigated in the present study.

| **Farm** | **N° of isolates** | **Municipality** | **Phenotypic resistance** |
|---|---|---|---|
| A | 2 | Barbacena | Amp[1], Pen[2], Tet[3] |
| B | 9 | Bias Fortes | Amp, Pen |
| C | 7 | Santa Rita de Ibitipoca | Amp, Pen, Tet |
| D | 11 | Santa Rita de Ibitipoca | Amp, Pen, Tet |
| E | 5 | Santa Rita de Ibitipoca | Amp, Pen, Tet |
| F | 16 | Lima Duarte | Amp, Pen, Tet |
| G | 15 | Rio Preto | Amp, Pen, Tet |
| H | 15 | Rio Preto | Amp, Pen, Tet |
| I | 6 | Rio Preto | Amp, Pen, Tet |
| J | 4 | Bicas | Amp, Pen, Tet |

[1]Amp: ampicillin; [2]Pen: penicillin; [3]Tet: tetracycline.

Supplementary Table S2. Results of the tests performed for the characterization of ampicillin and penicillin resistance in *Staphylococcus aureus* (n = 90) isolated from bovine mastitis.

| ***blaZ*** | **MIC[1] AMP[2]/PEN[3]** | **MIC range AMP/PEN (µg/mL)** | **Beta-lactamase production** | **N° of isolates** |
|---|---|---|---|---|
| + | R[4]/R | 0.5-4/0.25-8 | +[6] | 69 |
| + | S[5]/R | 0.25/0.25 | + | 6 |
| + | S/S | 0,125-0.25/0.06-0.125 | + | 3 |
| + | S/S | 0.125-0.25/0.03-0.125 | -[7] | 10 |
| - | S/S | 0.06-0.125/0.125 | - | 2 |
| **Total** | | | | **90** |

[1]MIC=Minimum inhibitory concentration; [2]AMP=ampicillin; [3]PEN=penicillin; [4]R=resistant; S=susceptible; [6] + = positive; [7] - = negative.

Supplementary Table S3. Results of the tests performed for the characterization of tetracycline resistance in *Staphylococcus aureus* (n = 90) isolated from bovine mastitis.

| **Gene** | **MIC[1]** | **MIC range (µg/mL)** | **N° of isolates** |
|---|---|---|---|
| ***tet*(K)** | R[2] | 16-128 | 56 |
| ***tet*(K)** | S[3] | 0.25-8 | 15 |
| ***tet*(L)** | R | 64-128 | 3 |
| ***tet*(L)** | S | 1 | 1 |
| ***tet*(M)** | R | 256 | 1 |
| ***tet*(K)(L)** | R | 32-64 | 3 |
| ***tet*(K)(L)** | S | 0.5 | 1 |
| ***tet*(K)(M)(O)** | R | 32 | 1 |
| **-** | S | 0.5-1 | 9 |
| **Total** | | | **90** |

[1]MIC=Minimum inhibitory concentration; [2]R=resistant; [3]S=susceptible