

Multiplex-PCR for detection of beta-lactam resistance in *Staphylococcus* spp.

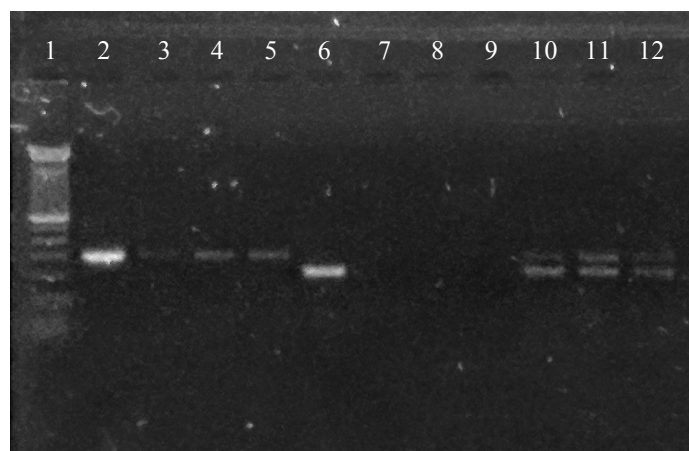


Figure 1. Electrophoresis of agarose gel stained with SYBR[®] Safe of the PCR Multiplex Standardization. **Line 1:** ladder of 100 bp (Invitrogen[®]); **Line 2:** *mecA* primers in the ATCC 43300; **Line 3 to 5:** *mecA* primers in the *Staphylococcus schleiferi* - 52° C, 54° C, 56° C; **Line 6:** 16S primers in the ATCC 43300; **Line 7 to 9:** 16S primers in the *Staphylococcus schleiferi* - 52° C, 54° C, 56° C; **Line 10:** *mecA* and 16S primers in the *Staphylococcus schleiferi* - 52° C, 54° C, 56° C; **Line 2 and 6:** 40 cycles of 94° C for 1 min., 54° C for 1 min. and 72° C for 1 min.; **Lines 3 to 5 and 7 to 12:** 30 cycles of 94° C for 30 seg., 52/54/56° C for 30 seg. and 72° C for 30 seg.

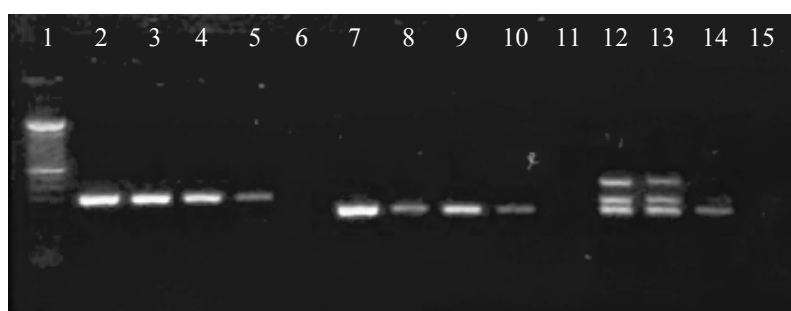


Figure 2. Electrophoresis of agarose gel stained with SYBR[®] Safe of the PCR Multiplex Standardization. **Line 1:** ladder of 100 bp (Invitrogen[®]); **Line 2 and 7:** ATCC 43300; **Line 3 to 6 and 8 to 15:** *Staphylococcus schleiferi*; **Line 3 to 6:** *mecA* primers; **Line 7 to 11:** 16S primers; **Line 12 to 15:** *mecA* and 16S primers; **Line 3, 8 and 12:** 0.4 pmol of primer, 0.4 mM of dNTP, 2 Mm of MgCl₂ and 1.25 U of polimerase; **Line 4, 9 and 13:** 0,2 pmol of primer; 0,3 mM of dNTP; 1,5 mM of MgCl₂; 1U of polimerase; **Line 5, 10 and 14:** 0,1 pmol of primer; 0,2 mM of dNTP; 1 mM MgCl₂; 0,5U of polimerase; **Line 6, 11 and 15:** 0,05 pmol de primer; 0,1 mM of dNTP; 0,5 mM of MgCl₂; 0,25U of polimerase.

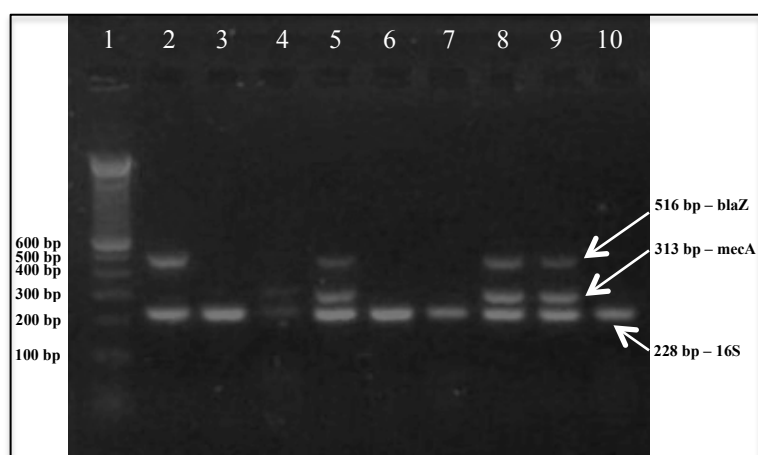


Figure 3. Electrophoresis of agarose gel stained with SYBR[®] Safe of amplified products of Multiplex PCR for beta-lactam resistance in *Staphylococcus pseudintermedius*. **Line 1:** ladder of 100 bp; **Line 2:** blaZ-positive with MIC 0.5 ug/ml; **Line 3:** beta-lactam susceptible with MIC 0.5 ug/ml; **Line 4:** mecA-positive with MIC 32 ug/mL; **Line 5:** mecA and blaZ-positive with MIC 32 ug/mL; **Line 6 and 7:** beta-lactam susceptible with MIC 0.5 ug/ml; **Line 8:** mecA and blaZ-positive with MIC 64 ug/mL; **Line 9:** mecA and blaZ-positive with MIC 8 ug/mL; **Line 10:** beta-lactam susceptible with MIC 0.5 ug/ml.

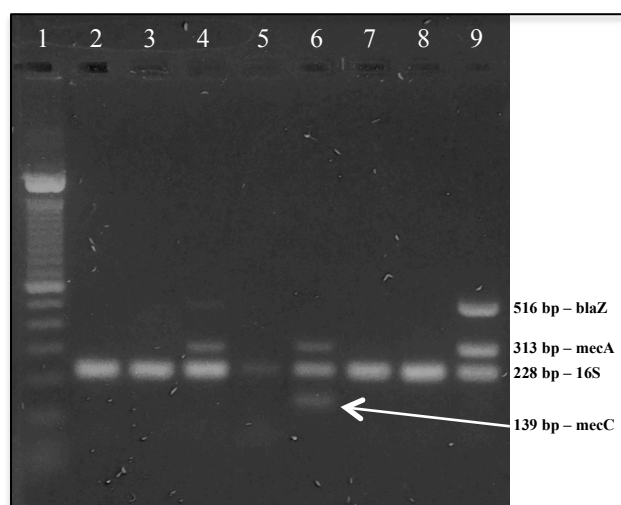


Figure 4. Electrophoresis of agarose gel stained with SYBR[®] Safe of amplified products of Multiplex PCR for beta-lactam resistance in *Staphylococcus* spp. **Line 1:** ladder of 100 bp; **Line 2, 3, 5, 7 and 8:** beta-lactam susceptible; **Line 4 and 9:** mecA and blaZ-positive; **Line 6:** mecA and mecC-positive.

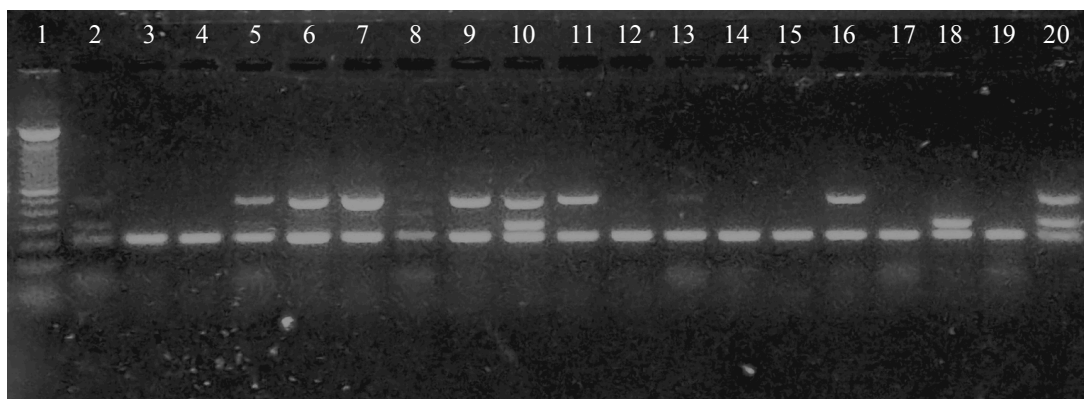


Figure 5. Electrophoresis of agarose gel stained with SYBR[®] Safe of amplified products of Multiplex PCR for beta-lactam resistance in *Staphylococcus* spp. **Line 1:** ladder of 100 bp; **Line 3, 4, 8, 12, 14, 15, 17 and 19:** beta-lactam susceptible; **Line 2, 10 and 20:** mecA and blaZ-positive; **Line 18:** mecA-positive; **Line 5, 6, 7, 9, 11, 13, 16:** blaZ-positive.

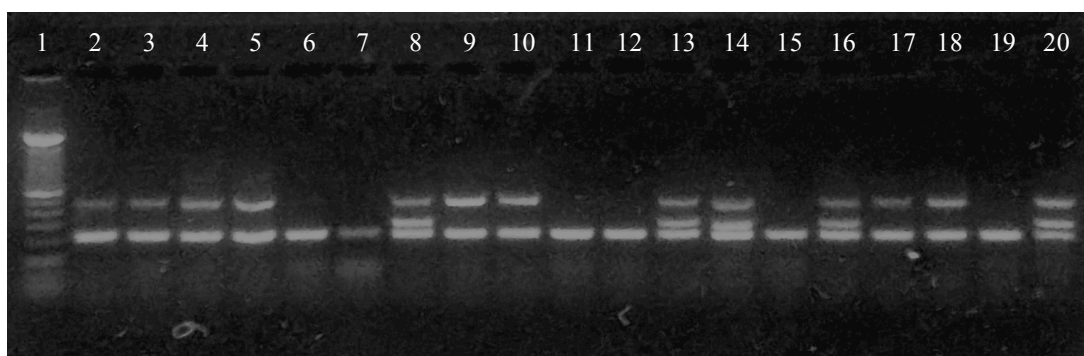


Figure 6. Electrophoresis of agarose gel stained with SYBR[®] Safe of amplified products of Multiplex PCR for beta-lactam resistance in *Staphylococcus* spp. **Line 1:** ladder of 100 bp; **Line 6, 7, 11, 12, 15 and 19:** beta-lactam susceptible; **Line 8, 13, 14, 16 and 20:** mecA and blaZ-positive; **Line 2, 3, 4, 5, 9, 10, 17 and 18:** blaZ-positive.