29th ECCMID Amsterdam, Netherlands 13 – 16 April 2019

The congress of ESCMID

O0927 The genetic background of MRSA clinical isolates interferes with their ability to invade and persist into human osteoblasts

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Background: The host-pathogen interaction is dynamic and requires several changes to promote bacterial survival. All infection and post-invasion effects are dependent on the interplay between different factors. This study comparatively analyzed the interaction and persistence of different well-characterized *Staphylococcus aureus* clinical strains belonging to the main ST-MRSA-SCC*mec* clones within human MG-63 osteoblasts, and preliminarily the modulation of the expression of virulence factors.

Materials/methods: To overcome the limitations of the *ex-vivo* model, the internalization frequency was evaluated at a multiplicity of infection of 100, for 16 MRSA isolates and ATCC12598, by Flowcytometric assay (Amnis FlowSight® Imaging) after lysostaphin treatment and vancomycin-Bodipy staining, to determine the copy number and persistence of intracellular bacteria, 24h after infection. The graph shows the correlation between internalization and the statistical significance (GraphPad Prism, $p<0.05$). The MG-63 viability was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide) reduction assay, at 2 and 24h post-infection. The *agr*, *hla*, *sig*B, *sar*A and *fnb*A/B expression levels were evaluated by RealTime-PCR (LC480 System, Roche), in intracellular condition model.

Results: The internalized bacteria were calculated counting the fluorescence spots inside MG-63 cells considering 10000 events. A statistical difference of internalization was found in ST5-SCC*mec*II and ST228-SCC*mec*I; ST239/241-SCC*mec*III, ST8-SCC*mec*IV and ST22-SCC*mec*IVh showed the same ability to internalized of the ATCC12598. Cell viability during the infection period showed a growth slowdown in ST5-SCC*mec*II strains at 24h and in a ST8-SCC*mec*IV at 2h. Preliminary data expressed as the mean of CT values compared with *gmk* showed a relative increase in gene expression of *agr*A and *sig*B, compared with housekeeping gene (*gmk*), and a relative decrease of *fnb*A/B.

Conclusions: Using Flowcytometric assay, we obtained greater reproducibility rate of internalization and number/spots of intracellular bacteria, using live cells and lower time/cost consuming. The passage from the extracellular to the intracellular behavior showed changes in the fibronectin-binding protein expression, important for host/cell invasion but heavy for intracellular persistence. The combination of these techniques allowed us to understand how the interaction between host and clinical strain varies among the diverse genetic backgrounds.