Title: Analysis of the virulence profile of community-associated and healthcare-associated methicillin-resistant *Staphylococcus aureus* using a *Caenorhabditis elegans* model

Authors: Bandeira, P. T.[1], Sant' anna , V. S.[2], Figueiredo, A. M.[1]

Institution: [1] Universidade Federal do Rio de Janeiro, Instituto de Microbiologia Prof. Paulo de Góes, Laboratório de Biologia Molecular de Bactérias, [2] Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho, Laboratório de Biologia de Helmintos Otto Wucherer

Abstract:

Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) have emerged as major human pathogens, and some studies have indicated an enhanced virulence for CA-MRSA in comparison with healthcare-associated MRSA (HA-MRSA). Recently, isolates closely related to the CA-MRSA clone USA400 (ST1-SCC*mec*IV lineage) have emerged in Rio de Janeiro strictly associated with nosocomial environment. The aim of this work was to use the roundworm *Caenorhabditis elegans* as a host model to compare the virulence of isolates within the same lineage (ST1-SCCmecIV) but some restricted to healthcare-associated (Brazilian isolates) and others to community-associated infections (American isolates). In addition, we used a well-characterized *S. aureus* mutant to validate this model as a useful tool to study the virulence in this species. The ST1-SCCmecIV isolates used in this study were 07-59 and 08-28 (HA-MRSA from Brazil), and USA400-0051 and MW2 (CA-MRSA from the EUA). We also evaluate the *S. aureus* mutant MNY19335 (a knockout in the global virulence regulator *agr*) and the isogenic wild-type NY19335, since there is a consensus that *agr* mutation attenuates virulence. The virulence was assessed in a blinded study using *C. elegans* survival assays and Kaplan-Meier survival method. The significance of the survival rates were calculated using the log-rank test. The nematode killing curves of the *agr* mutant and isogenic wild-type were significantly different (p value = 0.0048), confirming the more intense virulence phenotype for the wild-type strain NY19335, corroborating the accuracy of this model. No statistical significance was detected in the survival curve for the American CA-MRSA isolates, and both were highly pathogenic for the nematodes. Similarly, no significant difference in nematode killing was detected for the Brazilian HA-MRSA isolates. However, the Brazilian isolate 08-28 was less virulence than both American isolates, MW2 and USA400-0051 (p value < 0.0001 and p = 0.0036; respectively). In spite of the fact that the isolate 07-59 showed a reduced virulence in comparison with MW2 (p value of 0.0010), no statistical difference was detected when the survival curves of 07-59 and USA400 isolates were compared. These results confirm the highly virulent profile of CA-MRSA, supporting *C. elegans* as interesting host model to study the *S. aureus* virulence, and shows that the virulence of some HA-MRSA isolates may be variable when tested by this model.

Keywords: *Staphylococcus aureus*, ST1-SCC*mec*IV, USA400, CA-MRSA, HA-MRSA, *Caenorhabditis elegans*, Virulence host model

Sponsorship: FAPERJ, CNPq, CAPES