Importance of efflux systems on the resistance to fluoroquinolones in *Staphylococcus aureus*

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Staphylococcus aureus is an important clinical pathogen for which antimicrobial resistance mediated by efflux systems is still poorly characterized. Nevertheless, several efflux pumps have been described, which confer resistance to different types of compounds, including antibiotics, biocides and dyes, such as ethidium bromide (EtBr).

In order to understand the role played by efflux systems in the resistance to fluoroquinolones, we screened and characterized the efflux systems present in a collection of 52 *S. aureus* fluoroquinolones resistant clinical isolates.

The efflux activity was evaluated by the EtBr-agar cartwheel method and the determination of the minimum inhibitory concentrations (MICs) for different substrates of efflux pumps, including fluoroquinolones, in the presence and absence of efflux pumps inhibitors (EPIs). Genes coding for efflux pumps were screened by PCR and their expression level assessed by RT-qPCR. The presence of mutations that confer resistance to fluoroquinolones was examined by sequencing the QRDR of *gyrA* and *grlA* genes.

The application of these different methodologies detected efflux activity in 12 of the 52 isolates screened, and correlated this activity with overexpression of several efflux pump genes and increased resistance to fluoroquinolones. The modulation of the activity of these efflux systems by EPIs did not result in the total reversion of the resistance phenotype to susceptibility, yet it implied a significant decrease in the resistance levels to these antibiotics, regardless of the type(s) of mutation(s) found in *grlA* and / or *gyrA* genes. The mutations found in *grlA* and / or *gyrA* genes accounted for the remaining level of resistance to fluoroquinolones that was not efflux mediated.

Thus, the results obtained in this work do not exclude the importance of these mutations in resistance to fluoroquinolones in S. aureus, but underline the contribution of efflux systems for the emergence of high-level resistance to these drugs and eventually to the emergence of multidrug resistant *S. aureus* in hospitals.