

**TITLE:** PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF QUINOLONES AND ANTISEPTIC RESISTANCE AND PRESENCE OF EFFLUX PUMPS GENES IN DIFFERENT *Staphylococcus aureus* ISOLATES FROM HOSPITALS IN RIO DE JANEIRO

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**ABSTRACT:**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a frequent cause of nosocomial and community infections and fluoroquinolones can be used to treat these infections. Resistance to fluoroquinolones is characterized by the presence of mutations at *gyrA*, *gyrB*, *parC* and/or *parE* genes. Another mechanism of resistance is based on presence of efflux pumps. The expression of genes related to the efflux pumps has been associated with a high minimum inhibitory concentration (MIC) of fluoroquinolones, as well as antiseptic agents, such as chlorhexidine. The aim of this study was to identify the presence of efflux pumps to quinolones and sanitizing agents in MRSA isolates of prevalent and sporadic clonal lineages isolated from Rio de Janeiro hospitals. Eighty *S. aureus* isolates previously characterized regarding SCCmec typing, clonal lineages and antimicrobial resistance were evaluated to determine MIC for ciprofloxacin, moxifloxacin, and chlorhexidine by the microdilution broth method. Sequencing of PCR products amplified from genes related to efflux pump *norA*, *norB*, *norC* and *qacA/B* was performed. Phenotypic expression of efflux pumps was analyzed by the Ethidium Bromide-Agar Cartwheel Method (EtBrCW). Among the isolates tested, MIC<sub>50</sub> and MIC<sub>90</sub> for ciprofloxacin were 32 and  $\geq 256$   $\mu\text{g/mL}$ , while moxifloxacin MICs were 1 and 32  $\mu\text{g/mL}$ , respectively. Chlorhexidine MICs were 0.00048% and 0.00098%, respectively, and 74 (92.5%) isolates presented tolerance values ( $\geq 0.00024\%$ ). Among isolates tested, 18 (22.5%) were positive for active efflux pump systems (10% of them emitted fluorescence at  $\geq 2.0$   $\mu\text{g/mL}$  EtBr and 12.5% between 1.0  $\mu\text{g/mL}$  and 1.5  $\mu\text{g/mL}$ ). Among the eight isolates with the highest efflux potential, four (50%) belonged to the USA100/ST5 lineage. All the isolates were positive to *norA*, *norB* and *norC*, including isolates that were susceptible to ciprofloxacin and moxifloxacin. For 41 isolates tested, 28 (68.3%) were positive to *qacA/B* and 8 (19.5%) also had active efflux pump systems, while 20 (48.8%) were *qacA/B* positive and did not have these active systems. The preliminary data demonstrate that some *S. aureus* isolates have genes to efflux pump systems, but may not express this resistance/tolerance phenotype. Therefore, more studies are necessary to identify efflux pump systems presented by these isolates. MRSA isolates with active efflux systems seem to be associated with specific lineages, highlighting the importance of epidemiological studies in this context.

**KEYWORDS:** MRSA, efflux pumps, fluoroquinolones, *S. aureus*, chlorhexidine

**DEVELOPMENT AGENCY:** CAPES, CNPq, FAPERJ