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Whole transcriptomic analysis of chlorhexidine-tolerance mechanisms in Acinetobacter baumannii ST-2 clinical strain

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Background: Chlorhexidine (CHX) is a broad-spectrum antimicrobial agent with wide application. Nonetheless, a number of microbial nosocomial pathogens display high-level tolerance to CHX. For example, *A.baumannii* isolates from some health care settings can survive CHX concentrations of at least 1%. However, very little is known of the underlying mechanisms for this tolerance. In this work, a mutant chlorhexidine-tolerant *A. baumannii* (70S+CHX; MIC=625 mg/L) was obtained from *A. baumannii* ST-2 clinical strain (70 SIB; MIC=20 mg/L). We then used transcriptomic analysis by CHX tolerant and CHX susceptible in the presence and absence of CHX in order to examine the adaptive response.

Material/methods: We worked with 4 replicates of RNA total from 70S+CHX in comparison with 70 SIB in presence of 1/4 of the MIC to CHX. The quality of the RNA samples was studied with Bioanalyzer 2100. Transcriptome libraries were carried out using Ion Total RNA-Seq Kit v2 and Ion Xpress™ The FASTQ files were generated from the resulting readings (>80 millions) by FileExporter 4.6.0.0 plugin. Functional annotation of each predicted protein was developed with Blast2Go and InterProScan. Moreover, genes from mechanisms of interest (as Quorum Sensing system, [QS]) were studied by RT-PCR. The gene was overexpressed when FC ≥1.5. Finally, we analyzed the QS phenotypic through the biofilm quantification studies (Álvarez-Fraga L et al., 2016).

Results: The total differentially expressed genes between 70S+CHX and 70 SIB identified by edgeR and DESeq2 methods were of 1685 *versus* 1204. We found overexpression of genes in 70S+CHX in relation with rhizome: i) RESISTOME: AdeABC efflux pump (Fold Change [FC] 4.25 to 6.93), Arsenite efflux pump (FC 5.37), Tetracycline resistance protein (FC 6.11) and AceI-Chlorhexidine efflux pump (FC 3.6); ii) MOBILOME: Genes from plasmid AbATCC329 plasmid (PMMCU3p) as OXA 24/40 ß-lactamase (FC 12.16), DNA replication protein (FC 11.30) and OriV (FC 5.32) and, finally iii) PERSISTOME: All CsuABCDE operon (FC 2.57 to 3.51) implicated with biofilm formation and 10 proteins associated with the synthesis of polyketide antibiotics, including antibiotic biosynthesis monooxigenase (FC 2.19). Moreover, we found up-regulation of the *abaI* (FC 5.69) and *abaR* (FC 5.46) by RT-PCR indicating the QS activation. Finally, the 70S+CHX strain showed a higher biofilm formation than in the 70 SIB (Figures 1 and 2).

Conclusions: Therefore, the CHX tolerance in *A.baumannii* ST-2 clinical strain is an outcome of the effects of various mechanisms associated to changes in the resistome, mobilome and persistome, possibly associated with the activation of the QS.

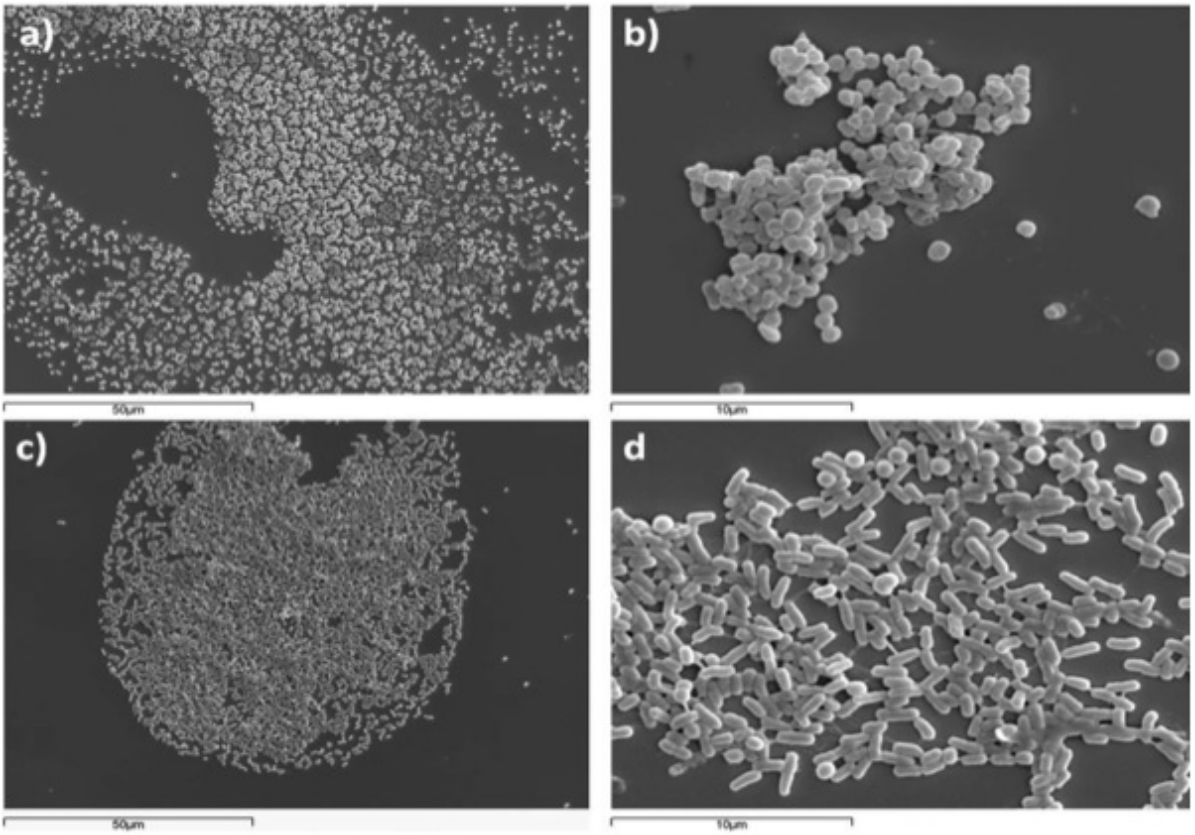

Figure 1

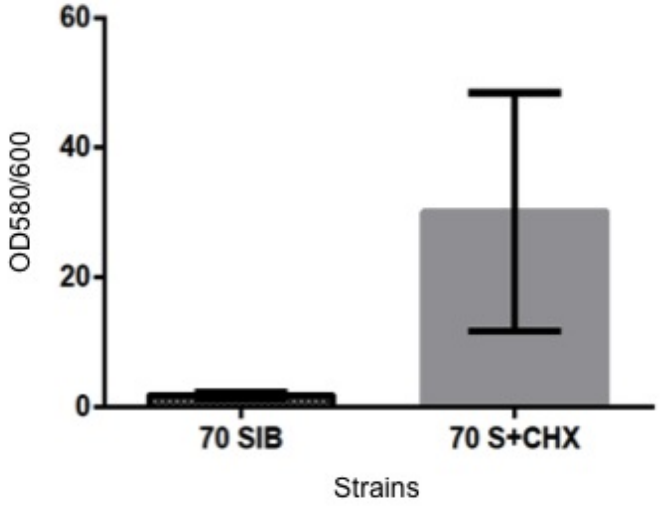

Figure 2

Figure 1. We can observe microcolonies (slime layer) from biofilm formation at 24 hours (a and b) by Scanning Electron Microscopy (SEM) studies in the 70S+CHX strain (tolerance mutant).

Figure 2. There was a significant increase of the biofilm formation through quantitative assays (Student´s t-test, $P<0.05$) in the 70S+CHX strain.