Evaluation of bacterial internalization using qRT-PCR

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Bacterial resistance to antibiotics currently represents a topic of high interest. Unfortunately, studies addressing their adaptation and multidrug tolerance latency¹ are not sufficient for a comprehensive description of the pathogen-host molecular interaction mechanisms (interactome) and the bacterial internalization into human cells, which leads to modified human genes. Bacterial persisters² are antibiotic-tolerant cells and their resistance can be better understood by exploring their growth status, signals and pathways leading to their formation in infected tissues. Some bacteria, as intracellular pathogens, directly interfere with transcription, translation, and DNA repair. Since metagenomic techniques were used to characterize the human microbiome,³ as in Human Microbiome Project⁴ and MetaHIT,⁵ both conducted between 2008-2012, several research projects emerged and all bacterial genomes detected in the human body can be consulted in the Genomes OnLine Database.⁶

Although culturing techniques still represent the gold standard for bacterial identification, PCR is a valuable tool for detecting bacterial pathogenic agents, especially when they die or lyse easily due to inappropriate storage conditions or prior antibiotic treatment, as it does not require live or intact cells.⁷ In clinical applications, real-time PCR for broad-range amplification of bacterial DNA offers a rapid turnaround time and a decreased risk of PCR carryover contamination, of outmost importance

when using small and qualitative-poor DNA samples. Furthermore, quantitative real-time reverse-transcription PCR (qRT-PCR) can be used to evaluate transcription⁸ and to measure gene expression levels in different mutant strains,⁹ offering a complete and realistic picture of the bacterial internalization.

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Conflicts of interest None to declare.

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