TITLE: GENE EXPRESSION PROFILE OF PENICILLIN-REFRACTORY *Streptococcus pyogenes* INDUCED AT HIGH-CELL DENSITY REVEALS SOME FEATURES ASSOCIATED WITH PERSISTERS

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ABSTRACT:

Streptococcus pyogenes are responsible for different types of infections ranging from uncomplicated pharyngitis to severe necrotizing infections. Despite these bacteria remain susceptible to penicillin (PEN), failures in antibiotic therapy have been reported and this phenomenon has not been clarified thus far. Previous studies of our group indicated that *S. pyogenes* exhibit refractory phenotype to different antibiotics, including PEN when grown at high-cell density (HCD; UFC/mL $>10^9$). The present work aimed to understand the molecular mechanisms involved in the emergence of HCD-induced antibiotic refractory. The transcript levels of 25 loci associated with efflux pumps, transport, cell metabolism, stress and also transcriptional regulators were analyzed by RT-qPCR using total RNA recovered from PEN-refractory cells, and also from antibiotic non-refractory *S. pyogenes* grown at low cell-density (LCD; UFC/mL $=10^7$). A significant increased level of transcripts (>4-fold) were observed in the PEN-refractory cells for the locus *MGAS10750_Spy1819* (gene product: RND efflux pump component HlyD) and of about 2-fold for the loci *MGAS10750_Spy0495* (multidrug resistance protein B), *norA* (transporter MFS superfamily) and *MGAS10750_Spy0043* (Na^+ driven multidrug efflux pump) compared with the antibiotic non-refractory *S. pyogenes*. It is impressive that the efflux pump repressor gene *MGAS10750_Spy1765* (MarR family transcriptional regulator) was down-regulated in the PEN-refractory cells, coherent with the increased expression of efflux pump-related genes. The gene *guaA* is important in the GTP synthesis. It has been shown that the decrease in the GTP metabolism is a target pathway for persisters since it leads to an increased accumulation of ppGpp, resulting in growth arrest and increased bacterial persistence. Consistently, the *guaA* mRNA decreased (>2-fold) in the PEN-refractory *S. pyogenes* compared with the non-refractory cells. In addition, other genes related with metabolism and cell division are down-regulated in the PEN-refractory cells. We have also grown *S. pyogenes* under stress condition such as acidic (pH 5.0) or low-iron (0.5mM 2,2-dipyridyl) environments. However, these conditions did not induce PEN-refractory cells, suggesting that this induction is HCD-dependent rather than stress associated. All together, these data indicate that PEN-refractory cells induced by growth at HCD share some features associated with persisters described in *E. coli* and *S. aureus*.

Keywords: *Steptococcus pyogenes***,** therapeutic failure, penicillin, persisters.
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