TITLE: EmrR-DEPENDENT UPREGULATION OF THE EFFLUX PUMP EmrCAB CONTRIBUTES TO ANTIBIOTIC RESISTANCE IN *Chromobacterium violaceum*

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ABSTRACT:

Chromobacterium violaceum is a ubiquitous environmental Gram-negative bacterium that causes rare but deadly infections in humans. Treatment of *C. violaceum* infections is difficult and little is known about the mechanisms of antibiotic resistance in this opportunistic pathogen. In this work, we identified mutations in the MarR family transcription factor EmrR and in the protein GyrA as key determinants of quinolone resistance in *C. violaceum*. Northern blot and EMSA assays revealed that EmrR directly represses the operon *emrCAB*, which encodes the major facilitator superfamily (MFS) tripartite efflux pump EmrCAB. Null deletion of *emrR* caused overexpression of *emrCAB* and increased resistance to nalidixic acid, but not to other quinolones or antibiotics of different classes. Additionally, the *emrR* mutant showed decreased production of the purple pigment violacein in liquid cultures. Complementation of the *emrR* mutant strain reverted nalidixic acid susceptibility, as determined by disk diffusion and minimum inhibitory concentration (MIC) assays, and restored violacein production. DNA microarray analyses indicated that, in addition to *emrCAB*, EmrR repressed other genes, some of them encoding putative transporters. To verify whether point mutation arise from EmrR and its contribution to quinolone resistance, we isolated *C. violaceum* spontaneous nalidixic acid-resistant mutants. We selected clones isolated from one to six-fold the MIC for nalidixic acid. Spontaneous mutants with high MIC values showed increased resistance to other quinolones (levofloxacin, ciprofloxacin and norfloxacin) and presented point mutations in the quinolone resistance–determining region (QRDR) of the gene *gyrA*, as determined by DNA sequencing. Otherwise, the spontaneous mutants with lower MIC values were more resistant only to nalidixic acid and two of them presented point mutations in EmrR. In conclusion, we determined that overexpression of the efflux pump EmrCAB and point mutation in GyrA confer distinct levels of quinolone resistance in *C. violaceum*.

Keywords: *Chromobacterium violaceum*, MarR transcription factors, antibiotic resistance; quinolone resistance, drug efflux pumps

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