Comparative subproteomic analysis of experimentally induced fluoroquinolone resistance and ciprofloxacin stress in Salmonella Typhimurium DT104B

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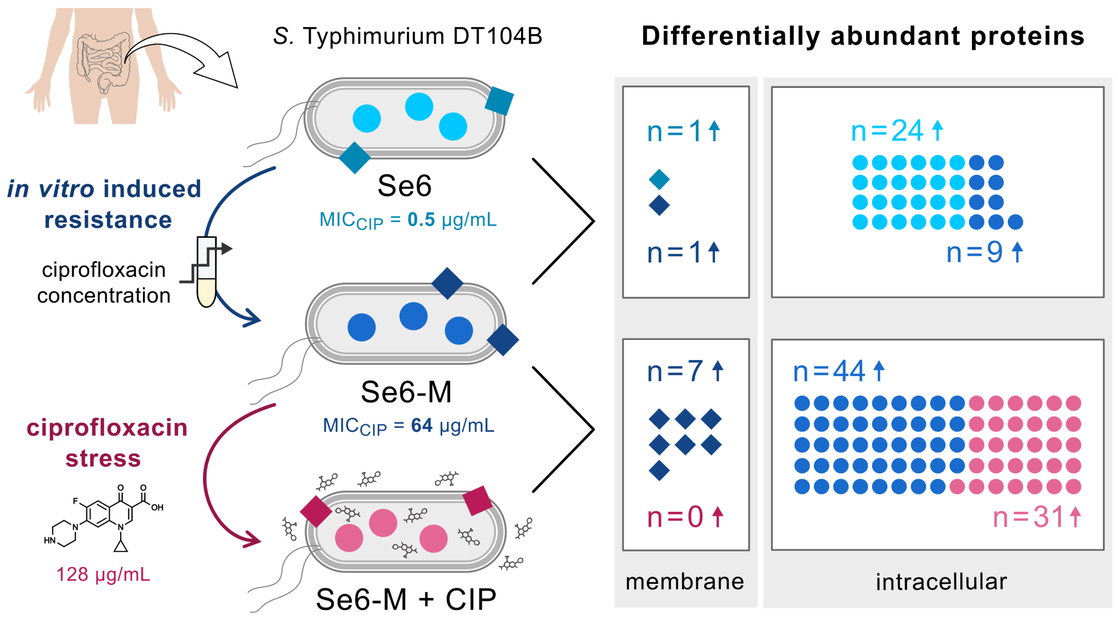
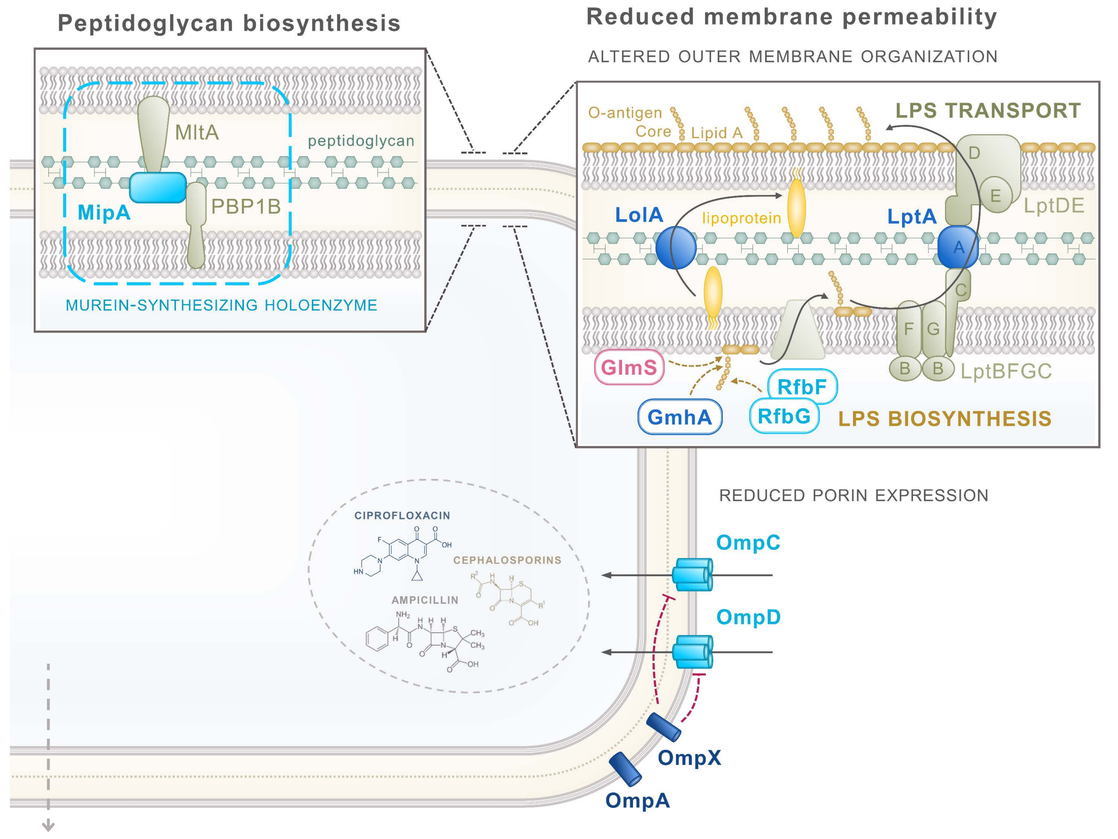
Fluoroquinolone-resistant *Salmonella* spp. is a global high priority pathogen for research and development of new antibiotics (World Health Organization, 2017) and *Salmonella* Typhimurium DT104B multiresistant strains with additional quinolone resistance have been responsible for global outbreaks and high mortality. Multiple factors are known to be involved in fluoroquinolone resistance; however, additional determinants continue to be described and the exact contribution of many mechanisms remains unexplained and/or need better understanding.

To give new insights into the mechanisms involved in fluoroquinolone resistance in *Salmonella* Typhimurium DT104B, this work aimed to evaluate subproteome changes between a laboratory-induced fluoroquinolone-resistant strain (Se6-M) and its parent strain (Se6) and also in Se6-M under ciprofloxacin (CIP) stress.

- A *Salmonella* Typhimurium DT104B isolate (Se6) was previously recovered from a hospitalized elderly patient with acute gastroenteritis before CIP treatment [1].
- An *in vitro* selected fluoroquinolone-resistant mutant (Se6-M) was experimentally obtained from the parental Se6 strain by a multi-step selecting process in the presence of increasing concentrations of CIP [2].
- Se6 and Se6-M were proven to be clonally related by PFGE.
- The different comparative subproteomic analyses were performed by combining 2-DE~LC-MSMS and shotgun LC-MSMS identification approaches for intracellular and membrane subproteomes, respectively.
- Proteomics data was validated by performing quantitative real time PCR for selected proteins of interest.



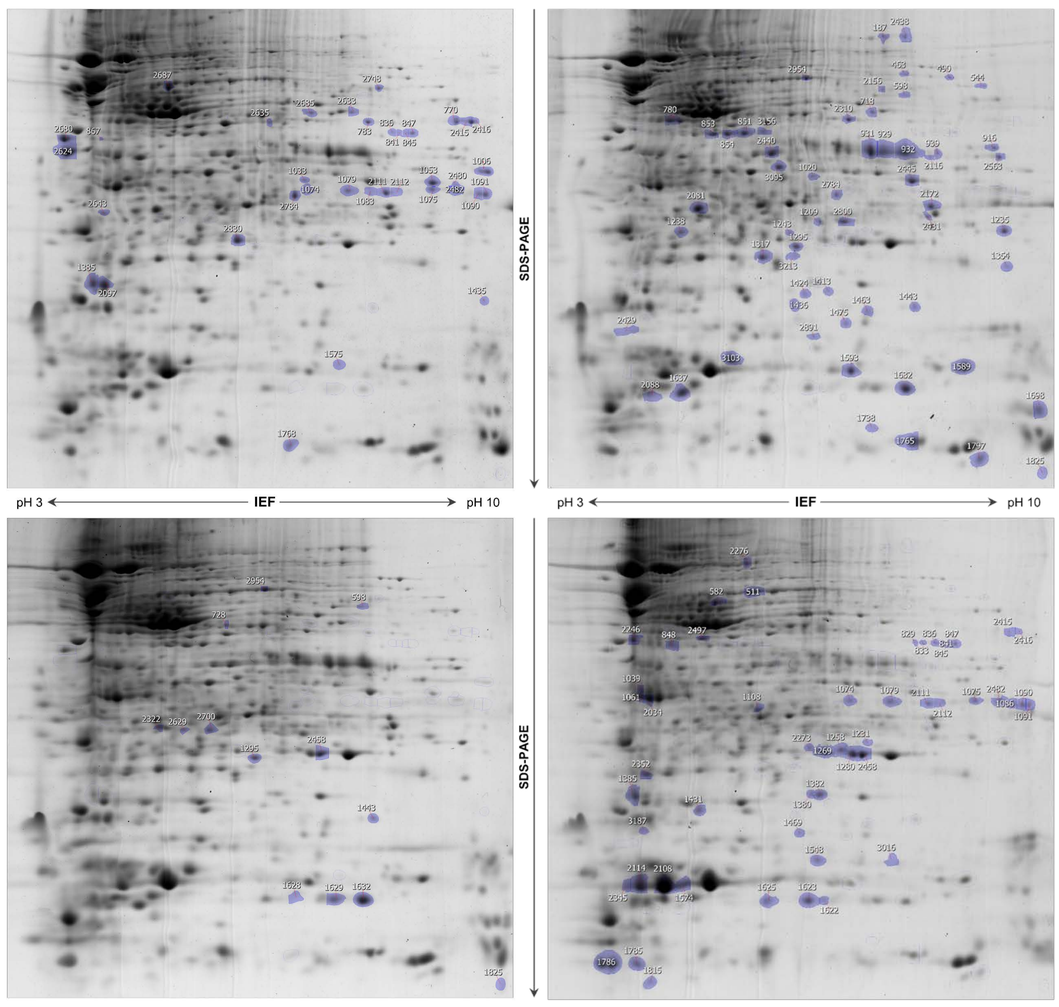
SCHEMATIC REPRESENTATION OF THE MAIN RESISTANCE MECHANISMS IDENTIFIED



Periplasmic substrate-binding proteins of ABC importers:
ArtI, FliY, GltI/GltI, GsiB, LsrB, MalE, ModA, MppA, Oxd-6a, PstS, RbsB, UgpB, YrbC

Global regulators of bacterial metabolism and bacterial stress response proteins:
CspA, CspC, H-NS, IhfA, LexA, NusG, PhoP, RecA, Rho, SeqA, StpA,

Roles as determinants of antimicrobial resistance and/or targets for the development of new antimicrobial drugs



Representative 2-DE gels with the protein spots identified by LC-MSMS with significant differential abundance between the intracellular subproteomes of the *S.* Typhimurium DT104B strains (SameSpots software). Left: the protein spots more abundant in Se6 are represented on top and the more abundant in Se6-M are shown on the bottom. Right: the protein spots more abundant in Se6-M grown without ciprofloxacin stress are represented on top and the more abundant in Se20+CIP are shown on the bottom.

The proteomic signatures obtained provide valuable information about mechanism-related differential protein expression, supporting the current knowledge and leading to new testable hypotheses on the mechanisms of bacterial resistance to fluoroquinolones and other drugs.

[1] de Toro M, et al (2010). *In vivo* selection of *aac(6')-Ib-cr* and mutations in the *gyrA* gene in a clinical *qnrS1*-positive *Salmonella enterica* serovar Typhimurium DT104B strain recovered after fluoroquinolone treatment. *J Antimicrob Chemother* 65(9): 1945-1949.

[2] Correia S, et al (2016). Impacts of experimentally induced and clinically acquired quinolone resistance on the membrane and intracellular subproteomes of *Salmonella* Typhimurium DT104B. *J Proteomics* 145: 46-59.

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