LC-MS/MS proteomics for the rapid and selective screening of drug resistances in *Leishmania infantum* clinical isolates

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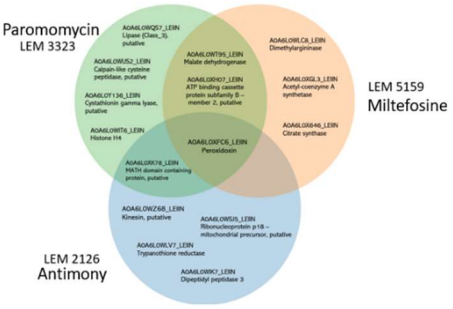

Vector borne diseases (VDBs) are the cause of more than 75% of the emerging human infections worldwide originated from animals. Severe changes in the atmospheric climate conditions, along with the upcoming of globalization and world-wide trade exchanges, have contributed to the spread of this infection outside the African Continent to the south European areas. Mass Spectrometry (MS) studies to rapidly detect bacterial infections and drug resistance in the human/animal bodies and environment, can also be proposed for the detection of rapidly incoming and less known parasitic diseases. One of the most diffused VDB is represented by Leishmaniasis, represented by over 12m of new clinical cases every year worldwide afflicting both humans and dogs, one third of which are hit by recrudescence during their therapeutic treatment. Depending on the endemic region of diffusion and the specific Leishmania strain considered, current therapeutic options include miltefosine, antimonials and paromomycin. The unsupervised usage of those or similar drugs in the livestock and humans has selected specific hyper-resistant strains, determining the rapid onset of severe drug resistant issues. This leads to decrease of drugs efficacy and increase of the risk of diffusion of the infections.[1] For this reason, in the present work we investigate through MS the potential application of an highly sensitive assay based on the study of proteome modulation of different Leishmania infantum strains characterized by drug resistance using as a model, THP-1 cells *in vitro*. We have treated the THP-1 cell lysates with a well-established FASP protocol to extract and hydrolyse proteins, which were analysed with LC-MS/MS bottom-up proteomics[2]. The quali-quantitative differential analysis of the samples performed with Mascot and Progenesis (Waters) against sample controls (non-resistant lines) revealed the presence of 15 differentially Expressed Proteins (DEP's), 6 of which in miltefosine sample, 8 in paromomycin and 6 in Sb(V) resistant strain. Some DEPs are mutual to more than one lines, and peroxidoxin - whose role in parasitic oxidative stress neutralization is well established - resulted up-regulated (FC >2) in all the three resistant lines. The modulation of these proteins could exploit the rapid determination of drug resistances patterns from clinical patients, to correctly evaluate the most promising therapeutic regimen and avoid the genetic selection of further resistances. Also, these biomarkers could be extended to dogs infected with Leishmaniasis for an early detection of the infection. Another important outcome of the present work is the identification of perspective targets for new therapy against drug resistant parasites.

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[1] Kaye, P et al. Leishmaniasis: complexity at the host–pathogen interface. *Nat Rev Microbiol* **9**, 604–615 (**2011**).
[2] Tagliazucchi, L et al. Label free Mass spectrometry proteomics reveals different pathways modulated in THP-1 cells infected with therapeutic failure and drug resistance Leishmania infantum clinical isolates. Accepted on *ACS Inf Dis*, Jan **2023.**