The *Saccharomyces* Deletion Library, an expansive collection of single gene deletion mutants, provides a tool for rapid genome-wide screens to aid in functional genomic research. Recently, we utilised the collection in a drug sensitivity screen with miltefosine, an anti-leishmanial that also shows potent anti-fungal activity.

While miltefosine has been used clinically in the treatment of visceral leishmaniasis since the early 2000s its precise molecular target and mechanism of action remains unclear, with evidence pointing towards a complex and polypharmacological model. Here we attempt to use the genetic tools available in model yeasts such as *Saccharomyces cerevisiae* to accelerate mode of action studies on miltefosine in *Leishmania spp*. Utilising the yeast knockout collection allows the bypassing of common road-blocks in *Leishmania* mode of action studies, particularly the difficulty of targeted gene deletion in *Leishmania* and the dominance of drug transporters in resistance phenotypes. This approach aims to use an unbiased, genome-wide screen in *S. cerevisiae* to provide a starting point for further targeted study in *Leishmania mexicana* with a rational basis.

The screening of the yeast knockout collection highlighted key genes involved in both resistance and hyper-sensitivity to miltefosine, some already known and well-studied and others not previously recognised. GO term analysis on resistance-related genes revealed enrichment for genes involved in intracellular trafficking. Orthologues for key resistance-related genes were targeted for deletion in *Leishmania mexicana*, allowing for the building of a knockout library that can be investigated for drug sensitivity, phenotype, and fitness.