

The microbiome of insect vectors as hidden reservoirs for pathogens

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Insects are well-known mechanical and biological vectors of diseases. Flies such as blowflies and houseflies feed and breed in feces and decaying organic matter, where they may acquire a significant part of their microbiome. Likewise, mosquitoes can visit a variety of sites and acquire their microbiome from the environment and hosts where they feed. Flies and mosquitoes are ubiquitous and synanthropic, thus potentially playing an important role in the dispersal of microorganisms to humans, animals and plants. In this study, we investigated the microbiome of major insect vectors in different continents to great depth, including the Oriental latrine blowfly *Chrysomya megacephala*, the common housefly *Musca domestica* and the Asian tiger mosquito *Aedes albopictus*. In total, ~150 individual insects were sequenced through whole genome shotgun (WGS) and we performed an in silico approach to filter the host DNA sequences from datasets. This allowed for genomic and metagenomic analyses of the host-associated microbiome at the

species level. Identification of microorganisms was done with different bioinformatics approaches with varying stringencies. Different insect species segregate based on the microbial diversity and abundance, as shown with PCoA. Legs and wings of flies displayed the largest microbial diversity and were shown to be an important route for microbial dispersion. The environmental sequencing approach used detected a stochastic distribution of human pathogens in flies and mosquitoes. We were able to further analyse pathogens at genomic level, identifying virulence factors of the major causative agent of gastric ulcers, *Helicobacter pylori* and antimicrobial resistance in *Enterobacter cloacae* isolated from mosquitoes. Therefore, our metagenomic approach highlights the potential of insects in dispersing pathogens to humans, plants and animals through neglected epidemiological routes.

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