ABSTRACT

Malaria predominantly affects the African continent. The dominant African malaria vectors include mosquito species from the *Anopheles gambiae* complex and the *Anopheles funestus* group. Mosquito midgut microbiota play essential roles in physiology and disease transmission. Thus, if common bacteria exist amongst vector species, this could be exploited for malaria control. Accordingly, this study characterised the native midgut bacteria of *Anopheles arabiensis* (member of *An. gambiae* complex) and *Anopheles funestus* (member of *An. funestus* group). Additionally, field-collected mosquitoes are routinely preserved and transported to a laboratory for analysis and thus far, it is unknown if midgut bacteria can be identified from preserved *Anopheles*. Therefore, this study also investigated if midgut bacteria could be identified after mosquito preservation.

Midgut bacteria were identified using culture-dependent (midgut dissections, culturomics, MALDI-TOF MS) and culture-independent techniques (midgut dissections, bacterial DNA extraction, next generation sequencing). Bacteria were identified from colonised mosquitoes that were fresh (non-preserved) and preserved on silica desiccant or in RNA*later*™ solution. Bacteria were also characterised from field-collected *An. arabiensis* from KwaZulu-Natal and Mpumalanga.

Results show that midgut bacteria can be characterised from preserved *Anopheles* samples. *Aeromonas hydrophila*, *Elizabethkingia anophelis*, *Enterobacter cloacae*, *Herbaspirillum huttiense*, *Pseudomonas grimontii*, *Psychrobacter* species, *Raoultella ornithinolytica*, *Serratia oryzae*, and *Staphylococcus epidermidis* were bacteria isolated from both vectors. Of these, *S. epidermidis* was also identified in field-collected *An. arabiensis*, making this a promising bacterium for future studies investigating the use of mosquito midgut bacteria for disease control. Overall, this study characterised the common midgut bacteria between *An. arabiensis* and *An. funestus* and discovered that midgut bacteria could be identified from preserved *Anopheles*.