ABSTRACT

Enterobacter bugandensis belongs to the ESKAPE bacteria, a group notorious for being prominent causative agents of hospital-acquired infections. Despite being a recent discovery in 2016, *E. bugandens*is is an emerging pathogen that has been responsible for several bacteremia outbreaks. Notably, *E. bugandensis* possesses high levels of multidrug resistance and little is known about its pathogenesis, which complicates treatment. Gram-negative bacteria – including multiple ESKAPE pathogens – can employ type VI secretion systems (T6SS) for a range of functions, including pathogenesis, bacterial competition, and antifungal activity. The T6SS is a transmembrane nanomachine that functions like a bow-and-arrow to inject effectors (toxins) into neighboring cells. Previous findings from our lab indicate that type VI secretion systems (T6SSs) are widespread in *Enterobacter bugandensis* strains. Furthermore, our lab demonstrated that the T6SS is involved in bacterial competition and virulence in a model strain, *E. bugandensis* E104107. The investigation into the composition of the VgrG spike in this study necessitated the use of CRISPR technology to generate VgrG mutants. Attempts with lambda red mutagenesis were impeded by sustained cassette expression in the cells. The reintroduction of effector proteins into mutant *E. bugandensis* was accomplished successfully, as confirmed by protein secretion assays. Nonetheless, further research is required to assess the bacterial and virulence impacts of the reintroduced effector proteins.

Keywords: *Enterobacter bugandensis*, ESKAPE, multi-drug resistance, Type 6 secretion system, VgrG spike, mutagenesis.