I. INTRODUCTION

Enterobacter bugandensis is a Gram-negative bacteria that is part of the ESKAPE diseases and belongs to the Enterobacter genus (Mulani et al., 2019). It was found in 2016 during a nosocomial sepsis epidemic in a Tanzanian hospital and has been linked to many bacteremia outbreaks, with a particular impact on infants and people with weakened immune systems (Girlich et al., 2021). *E. bugandensis* has a high level of multidrug resistance and is thought to be the most virulent species in the *Enterobacter* genus. In the course of their activities, *E. bugandensis* employ secretion systems as a transportation strategies, enabling them to outcompete neighboring organisms and establish dominance in their surrounding environment (Wu et al., 2018). Gram-negative bacteria, in particular, utilize a variety of secretion systems, from types 1 to type 6. The specific combination of these systems within bacteria varies based on their behavior and lifestyle. *E.bugandensis* themselves, possess types 2, 4, and 6 secretion systems, each dedicated to transporting distinct substances.

Within *E. bugandensis*, the type 6 secretion system (T6SS) is of particular significance. The type 6 secretion system (T6SS) is a complex nano-contractile machine that span across a membrane complex, base plate complex, and the sheath and tube complex (Zoued et al., 2014). The mechanism operates base on contact-dependent manner and akin to a bow and arrow to initiate the firing of a puncturing device (Jana et al., 2022). This puncturing device is a complex composed of three monomers: VgrG 1, VgrG 2, and VgrG 3. Intriguingly, knocking out each VgrG gene individually suggest that the loss of one of the monomer options in forming the VgrG spike trimer does not impede T6SS secretion (Anderson, Unpublished). This raises questions about the potential heterotrimeric or homotrimeric composition within the VgrG spike complex. This device is also loaded with effector proteins that can be categorized into cargo and specialized effectors. Within the many effector proteins, our laboratory demonstrates that the deletion of 3 different individual genes, Tge 1, Tge 2 and Tge 3, markedly enhances the anti-virulence activity (Anderson, Unpublished), leading to interesting whether they can restore their effector protein to validate their virulence activity.

This study hope to investigate the structural configuration of the VgrG spike, assessing its potential influence on secretion by T6SS and to investigate the role of the Tge1, Tge2, and Tge3 effector candidate in virulence using bacterial competition and *G. mellonella* infection model. The research hypothesis is that the T6SS is involved in both bacterial competitiveness and virulence and that the specific effector candidates are important for the bacterium's pathogenicity. The goals of the study

are to bring Insight into *E. bugadensis* interaction with neighboring cells, providing potential novel mechanistic insights for the development of better therapeutic interventions.