Chapter 5

Conclusions and Future Prospects

5.1. Conclusions

E. cloacae have acquired clinical importance and emerged as a human opportunistic pathogen. It became one of the leading causes of nosocomial infections (hospital-acquired infections) around the globe. Its occurrence has further worsened due to the rise of resistance to antibiotic drugs in these bacteria, which makes the treatment difficult. Despite the relevance of *E. cloacae* as an opportunistic pathogen, very little is known about its pathogenicity mechanism and the factors influencing its virulence. Although there have been studies on the pathogenicity of a couple of clinical isolates, understanding of *E. cloacae* pathogenicity is not investigated in detail. Moreover, studying the pathogenic potential of exogenous (non-clinical) isolates remains ignored. Research in our laboratory identified a rhizospheric bacterial isolate obtained from the *Sorghum bicolor* plant grown at sambhar-lake of Rajasthan as *E. cloacae* SBP-8 (Accession No. NAIMCC-B-02025), , was. Detailed mining of the genome sequence of *E. cloacae* SBP-8 indicated the presence of T6SS, which isrecognized as a feature of pathogenic or clinical isolates of Gram-negative bacteria. However, it has not been studied and reported in opportunistic pathogens like *E. cloacae* of exogenous origin. Understanding the pathogenic potential and mechanisms of pathogenesis of such isolates is essential to contain the fatalities resulting from *E. cloacae* infections. Thus, we aimed to characterize the pathogenic potential of *E. cloacae* SBP-8 along with the role of T6SS in pathogenesis and bacterial competition.

We established *Caenorhabditis elegans* as a model host to test the pathogenicity of *E. cloacae* SBP-8. Our finding suggests that *E. cloacae* SBP-8 progressively colonized the intestine of *C. elegans,* which led to intestine distension and shortened the nematode life span. The bacterial colonization was localized in the animal by infecting GFP-tagged *E. cloacae* SBP-8 to the animal host followed by fluorescence microscopy. The bacterial infection caused physiological and reproductive defects in the infected worms. It included pharyngeal distension, and blockage of the vulval region leading to altered egg arrangement, internal egg hatching, and reduction in brood size. *E. cloacae* SBP-8 infection induced cell death (as assessed through DNA damage) and ROS production in the worms. RT-PCR-based gene expression analysisindicated stimulation of immune response by *E. cloacae* SBP-8 in worms by upregulating *tol-1*, a Toll-like receptor. The nematodes exhibited protective immune response by upregulating antimicrobial peptide genes, *lys-1*, *clec-60*, *clec-85,* and *clec-87.* However, these genes were downregulated at later hours (48 h), indicating the nematodes surrendered to the infection. A similar trend was observed for reproductive genes (*lin-29* and *let-23*), suggesting a struggle to maintain functional reproduction by the nematodes. These

results demonstrates that *E. cloacae* SBP-8 colonization in *C. elegans* affects its physiology, reproduction, and survival.

To gain insights into the systemic infection process of *E. cloacae* and to analyze different stages of bacterial infection, we used a murine model host. Our study shows that *E. cloacae* SBP-8 progressively colonized the reticuloendothelial organs like liver, lungs, spleen, and kidneys, when infected *via* the intra-peritoneal and intra-gastric route. *E. cloacae* SBP-8 infection elevated host innate immune response leading to the systemic production of proinflammatory cytokines. The infection induced recruitement of neutrophils and other innate immune cells to the kidneys and lungs. However, congestion, interalveolar septa thickening with mild fibrosis and infiltration of immune cell was observed only in lungs. Increased level of bile, AST/SGOT, AST/SGP in infected mice also indicated the liver damage. We also analyzed, if *E. cloacae* SBP-8 could inhibit or divert the normal process of phagosome maturation and withstand within the hostile environment of the phagosome using confocal microscopy. Based on our observation, we hypothesize that the most likely route of *E. cloacae* SBP-8 infection is feco-oral, whereby it crosses the intestinal epithelium and disseminates *via* phagocytes, at least the macrophages. In this study, we investigated various pathogenesis aspects using murine infection model, which can provide a helpful tool to gain insights into the pathogenesis of *E. cloacae*.

After confirming the pathogenicity of *E. cloacae* SBP-8 in animal model host, we intended to identify possible factor for pathogenesis. As the genome sequence analysis showed the presence of T6SS, one of the important factors in pathogenesis, in *E. cloacae* SBP-8, we characterized its role in pathogenesis of *E. cloacae* SBP-8. The *E. cloacae* SBP-8 genome shows a presence of one complete T6SS cluster, which is similar to the T6SS-1 cluster of *E. cloacae* ATCC 13047 (clinical isolates). Further, detection of Hcp effector protein in the secretome, confirmed that T6SS of *E. cloacae* SBP-8 is functional. As the secretion of Hcp is dependent on *clpV*, an ATPase of T6SS, we knocked out *clpV* to investigate role of T6SS. *E. cloacae* SBP-8 *ΔclpV* did not show any significant change in the life span and rate of colonization in *C. elegans* as compared to its wild type counterpart. Similarly, *E. cloacae* SBP-8 *ΔclpV* did not exhibit considerable difference in expression profiling of antimicrobial genes (*clec-60*, *clec-85*, *clec-87*, and *lys-1),* toll like receptor *(tol-1)* gene involved in stimulating an immune response against the pathogen. It also did not t affect the adhesion and proliferation of *E. cloacae* SBP-8 in eukaryotic cell lines. Based on these observations, T6SS appeard to have a little role in exerting pathogenesis caused by *E. cloacae* SBP-8. However, based on the result

of competition assay, it was inferred that *E. cloacae* SBP-8 employs T6SS for inter-bacterial competition and can outcompete neighoring bacteria in in contact-dependent manner. Since, the isolate is of environmental origin, T6SS required for pathogenicity could not be found in *E. cloacae* SBP-8. Overall, this study adds to our understanding of pathogenic potential of *E. cloacae* of exogenous origin and role of its T6SS in pathogenicity and inter-bacterial competition.

5.2. Future prospects

In the present work, we have established *C. elegans* and murine (mice) as a model host to study the pathogenicity of *E. cloacae* SBP-8 and identify the role of T6SS in its pathogenicity and bacterial competition. There are several aspects that could be studied in the future to understand the mechanisms of pathogenesis of such isolates in order to reduce fatalities and develop novel drug therapeutics for *E. cloacae* infections.

- Present study established *C. elegans* as an appropriate model organism for evalutating pathogenicity of bacteria. This can be extended for preliminary evaluation of pathogenicity in bacteria which has prospectively beneficial properties including plant growth promotion, bioremediation, and production of industrially useful compounds.
- We observed little effect of T6SS on pathogenicity in environmental isolate of *E. cloacae* unlike its clinical counterpart. Therefore, an extensive study can be carried out on T6SS of various clinical isolates of *E. cloacae* for in-depth understanding of its role in pathogenicity, if any. Further, its role in inter-bacterial competion as proven in our study and other related work, T6SS-containing bacteria can have implications on host-associated microflora, which in turn can lead to dysbiosis. Resultantly, it can ensue health related complications in the host.
- SDS-PAGE analysis of *E. cloacae* SBP-8 wild type and *E. cloacae* SBP-8 *ΔclpV* knockout strains show differential proteomic profile of secreted proteins. It indicates that ClpV may have global effect on gene expression/secretion in the bacteria which can be investigating by total transcriptomic profiling.