CHAPTER 7

SUMMARY

- SPATEs have been implicated in the pathogenesis of various diarrheagenic *E. coli* isolates, as well as the diverse pathotypes of ExPEC isolates including isolates causing UTI, cystitis, pyelonephritis. However, no study has yet been undertaken to explore the occurrence of SPATEs among *E. coli* isolates causing neonatal septicaemia. This study is the first to investigate the distribution of SPATEs and different subtypes of SPATEs among NSEC isolates.
- The remarkably higher prevalence of SPATEs among the NSEC isolates made it imperative to check the distribution of SPATEs among the fecal *E. coli* isolates from healthy neonates and environmental *E. coli* isolates from ground water resources. Our data revealed significantly higher prevalence (89%) of SPATEs compared to the fecal isolates (7.5%) and environmental isolates (3%). The PFGE results further ruled out the possibility of similar clones contributing to the high prevalence of SPATEs. These results together confirm the specific association of SPATEs with NSEC isolates.
- Vat was found to be the most prevalent SPATE among the NSEC isolates followed by Sat which is consistent with the previous reports which have evidenced the involvement of these two subtypes of SPATEs among the isolates causing diverse extraintestinal diseases. In our study, 51% of the septicemic isolates possessed Vat and 39% of isolates harbored Sat. The prevalence of other SPATEs was much lower, with EspP (13%), SepA (11%), Pet (10%), Pic (9%) and SigA (1%). None of the NSEC isolates were positive for the presence of Tsh (which is also named as Hbp), EatA and EspC.
- An analysis of the different phylogroups of *E. coli* isolates and distribution of SPATEs in those groups was performed. According to previous studies, ExPEC isolates mostly belong to phylogroup B2 and to a lesser extent to group D. In our

study, though 67% of septicemic isolates belong to groups B2 and D, what was interesting was the presence of 33% of A and B1 isolates. Furthermore, a large proportion of septicemic A and B1 isolates (87%) possessed SPATEs in our study. This actually gave us a hint of the role of SPATEs in the pathogenesis of NSEC isolates.

- Our data also showed that most of the isolates of phylogroup B2 (86%) harboured multiple subtypes of SPATEs. This further re-establishes the virulence potential of this phylogroup.
- An analysis of the most predominant combination of SPATEs showed that the specific combination of Vat and Sat is the most prevalent among the NSEC isolates.
- The presence of class I SPATEs appeared to be markedly higher than class II SPATEs (59% vs 14%) among the NSEC isolates.
- The expression of SPATEs could not be detected for some NSEC isolates in skim milk assay due to several reasons such as distinct substrate specificities of different subtypes of SPATEs and factors affecting secretion of SPATEs to the extracellular milieu.
- The significant association of SPATEs with the septicemic isolates led us to look for the presence of other VFs among these isolates. Apart from *iucC,* which is generally considered as a defensive VF, all other virulence determinants were predominantly isolated from phylogroup B2. The NSEC isolates of other phylogroups (A, B1, D) possessed few VFs though the prevalence of SPATEs was high in them. This makes SPATEs as the most discriminatory trait for the NSEC isolates .

- The animal experiment ruled out the contribution of *iucC* in active virulence as this gene was also evidenced in the fecal isolates of phylogroups A and B1. The pathogenic potential of the NSEC isolates of phylogroups A and B1 possessing SPATEs but no other VFs except *iucC* was also validated by the animal experiment.
- In an attempt to purify and identify a secreted protease from NSEC isolate, we identified YghJ as a secreted metalloprotease of NSEC isolate by MS/MS pepide sequencing. Further characterization of this protease revealed that YghJ is optimally active at pH 7 to 8 and at temperature of 37 to 40°C. We got YghJ to be proteolytically active against gelatin, AAPM-pNA oligopeptide substrate, but inactive against casein.
- The genotypic and phenotypic distribution of YghJ was determined. Seventy eight percent of NSEC isolates were found to possess *yghJ* gene compared to 54% in case of fecal *E. coli* isolates. This led us to evaluate the expression and secretion of YghJ among these two groups of *E. coli* isolates. Our data revealed that the expression and secretion of YghJ was significantly higher among the septicemic isolates compared to the fecal *E. coli* isolates (80% versus 33%). This actually reflects the involvement of YghJ in the virulence of NSEC isolates.
- Our study is the first to show that YghJ induced cytotoxicity by exhibiting clear changes in the cellular morphologies of Int407, HT-29 and HEK293 cell lines. Moreover, YghJ triggered the upregulation of specific proinflammatory cytokines from mouse macrophages and human intestinal epithelial cell lines and also down-regulated the production of anti-inflammatory cytokine.
- The determination of association of YghJ with SPATEs revealed the exclusive association of YghJ with the most prevalent SPATE among NSEC isolates, Vat.