SUMMARY AND CONCLUSION

The present study was carried out to evaluate the immunoprotective potential of K-antigen conjugate against experimental ascending pyelonephritis and if this immunization can modulate course of the disease. The salient findings of the study are summarized below:

1. Based on the previous literature, the uropathogenic strain of *Escherichia coli* 06:K13:H1 procured from Collaborative Centre for Reference and Research on Escherichia (WHO), Denmark was used. This strain was selected for the study, since, it expresses O, K and H antigens of uropathogenic interest, produces hemolysin and shows attack rate of 60-80% in experimental animals.

2. Since, the receptors on uroepithelium of BALB/c mice are similar to human uroepithelial cell receptors, these were used for ascending pyelonephritis model without any physiological manipulations of the urinary tract.

3. Ascending pyelonephritis was produced in the female BALB/c mice, where 100 µl of bacterial suspension (10⁹ CFU/ml) was inoculated in the bladder through a polyethylene catheter, intrauretherally. Organisms were recovered from the kidneys on 2, 4 and 7 post-infection days.

4. In order to have better expression of antigen of interest (K13 antigen) from *E. coli* 06:K13:H1, bacteria were grown in self designed laboratory scale 20 litre fermentor in DO broth at 37°C and pH 7.4. The cells were harvested at late exponential phase which was attained within 5-6 h of incubation.
5. The negatively charged K13 capsular polysaccharide antigen was isolated by detergent extraction method and sequential ethanol precipitation. The total yield of antigen was 5 mg/litre of broth.

6. Purification of K13 antigen on Sepharose-6B column and elution with phosphate buffer containing 0.2M NaCl showed a single broad peak at a distribution coefficient (Kav) of 0.27 in elution profile. Purified maternal contained negligible contamination of LPS, nucleic acid and proteins.

7. Purified K13 antigen was successfully coupled to purified diphtheria toxoid (Kav 0.55) by carbodiimide condensation reaction using adipic dihydrazide (ADH) as spacer molecule (multipoint attachment method).

8. A good protein-polysaccharide ratio (1.86) and yield of coupled material (39.5%) was achieved through this method of conjugating the K13 antigen to diphtheria toxoid.

9. In contrast to unconjugated K13 antigen, K13-DT conjugate showed a good immunogenicity in mice, when injected subcutaneously. High titres of serum anti K13 antibodies were observed after 3 doses of K13-DT conjugate at an interval of 2 weeks. Immunization with K13-DT conjugate produced an appreciable booster response in the animals (T-dependency).

10. Adsorption of K13-DT conjugate on adjuvant (aluminium phosphate) enhanced the immunogenicity of conjugate in experimental mice.
Summary and Conclusion

11. Out of the many preparations of K13 antigens tested in relation to their immunogenicity and protective efficacy against experimental ascending pyelonephritis, the following observations were made in the K13-DT immunized-infected animals.
   i) There was a rapid clearance of bacteria from the kidney in this group.
   ii) Severity scores of pathological lesions were significantly lower than in the animals immunized with unconjugated K13 antigen and control animals (PBS-immunized).

12. To define the role of leucocytes in relation to specific and nonspecific immunity in protection as well as in tissue destruction, various immunological and biochemical parameters were studied in immunized and control animals as listed below.
   i) The involvement of ROS in providing protection or tissue damage was studied through the measurement of chemiluminescence response (CL). Renal macrophages and neutrophils of K13-DT conjugate immunized-infected animals showed a significantly lesser CL response than the infected animals (PBS) following the infection. Differences in the CL responses were also noted in blood monocytes and neutrophils of these infected groups, but was lower than the one seen in the renal tissue macrophages and neutrophils.
   ii) Malondialdehyde is an index of damage caused by ROS. Significantly higher levels of MDA were recorded in renal tissue homogenate of infected groups (PBS immunized) in comparison to conjugate immunized infected groups.
   iii) Significant rise in the activity of few enzymes in renal tissue
homogenate viz. glutathione reductase, glucose-6-PO₄ dehydrogenase and lactate dehydrogenase which are important for production of energy for free radical generation was observed in the infected group (PBS immunized) than the control and K13-DT conjugate immunised-infected groups.

iv) Phagocytosis and killing of ingested bacteria by lysosomal enzymes was measured by *in vitro* bactericidal assay. Due to infection, the decrease observed in the bactericidal capacity of the renal monophages and neutrophils was lesser in K13-DT immunized-infected group than infected group (PBS immunized).

v) The immunization with K13-DT conjugate prevented suppression of cell mediated immune response to an extent that it partially protected the animals against ascending pyelonephritis. This response was seen by in-vitro lymphocyte blast transformation assay. In K13-DT immunized infected animals, no marked suppression of the lymphoblastogenic response to Con A and PHA was observed. There was marked suppression in infected group (PBS immunized) on both 4th and 7th post infection days.

vi) A good lymphoblastogenic response was shown by splenic lymphocytes of K13-DT immunized animals to both K13-DT conjugate and DT antigen. Even after the infection suppression observed in this group was much less in comparison to infected group (PBS immunized), as it did not respond at all to these specific antigens.
vii) The splenic lymphocyte subsets were measured in all the groups by flowcytometry analysis to find out the alteration in CMI in relation to expression of surface markers of T-lymphocytes. A decrease in the CD4⁺/CD8⁺ ratio was observed in infected animals (PBS immunized) on both 4 and 7 postinfection day. Immunization with K13-DT conjugate prevented the alteration in CD4⁺/CD8⁺ ratio, which was initially decreases on 4 postinfection day but was restored to normal on 7 postinfection day.

13. Based on the results of this study, it is concluded that immunization with K13-DT conjugate in mice provides partial protection against experimental ascending pyelonephritis induced by homologous strain of *E. coli* 06:K13:H1. This protection is mediated through activation of humoral as well as cell mediated immunity against the invading organism.