7.0 SUMMARY

Infection caused by *S. Typhi* remains an important public health problem, particularly in developing countries. Morbidity and mortality, attributable to typhoid fever, are increasing with the emergence and worldwide spread of multidrug resistant *S. Typhi* strains. As a consequence, there is renewed interest in understanding the epidemiology, diagnosis and treatment of typhoid fever. Public health authorities should largely make use of the available rapid, simple and reliable diagnostic tools of typhoid fever, especially in health units where culture technique is unavailable.

A total of 194 isolates of *S. Typhi* were obtained from various labs in Chennai during June 2003-August 2009 and studied. Of the 194 isolates, majority of the isolates were from females (53%).

The titre of *Salmonella Typhi* O and H agglutination was found to be 1:160 by Widal analysis for most of the isolates (110 isolates).

The isolates were grouped based on conventional and molecular methods. The biotyping based on sugar fermentation grouped the isolates into three (Biotype I, II and III); the Biotype I had majority of the isolates (182).

All the 194 isolates were tested with the 14 antibiotics that were routinely used to treat typhoid were employed for antibiogram analysis. 17 isolates were sensitive to all the 14 antibiotics. 127 isolates showed resistant to nalidixic acid alone, 32 isolates found to be resistant only to Nalidixic acid and nitrofurantoin. The dendrogram analysis of antibiogram also
grouped the isolates into 5 clusters. The dendrogram analysis showed that antibiogram could be employed for grouping the isolates.

RAPD (Random Amplified Polymorphic DNA) method was used for the molecular typing of *Salmonella Typhi*. RAPD analysis was done using 3 different primers (A, B and C) and approximately 13 to 15 bands were obtained for each of these primers. RAPD analysis was able to group the isolates in wide range than conventional typing, thus confirming the potential of this technique.

New methods like dot-blot (using IgY against O, H and Vi antigens) and FTIR were also tested for the rapid diagnosis of *Salmonella Typhi* in this study. The polyclonal antibody (IgY) was produced in white leghorn chicken against the antigens of *S. Typhi* (O, H and Vi) the antibodies were purified and characterized using various immunological techniques like DID, CIE, etc.

Dot blot analysis was done and it had a good sensitivity as IgY (1:100) dilution was able to detect the organism or antigens even at low level. The cross-reactivity of these antibodies was tested by both dot and slot-blot methods where *S. Typhi, E. coli, Pseudomonas aeruginosa* and commercial *S. Typhi* antigens were tested. It was found that the polyclonal antibodies (IgY) raised against *S. Typhi* antigens (O, H and Vi) had no cross-reactivity as they detected only their corresponding antigens alone. This showed that the chicken antibodies could be a better alternative than IgG or IgM as large amounts of IgY could be obtained without sacrificing the animal and purification is also easy. Dot blot could be a better alternative than other molecular methods like PCR where DNA has to be isolated, since patient urine itself could be used as a source for detection of pathogen.
The patient serum (typhoid) and healthy serum were analysed using FTIR in this study. It was found that unique peaks were observed in the carbohydrate regions like 1076cm$^{-1}$, 1120cm$^{-1}$, 1540cm$^{-1}$ in the patient serum and could be used as marker for *S. Typhi*. Since this was a pilot study to test the applicability of FTIR only few samples were tested, a larger sample size would be providing more information.

The methanolic extract of *Swietienia mahagoni* was assayed for antibacterial activity against *S. Typhi*; an inhibition zone of 8 mm with 1000 µg/ml was observed. The bioautography data also correlated with the antibacterial activity assay. The MIC of the methanolic extract of SM3 on *S. Typhi* was found to be 17.5 mg. Different concentrations of the methanolic extract were tested for Brine shrimp lethality assay with two time intervals (24 and 48 hrs). The LC$_{50}$ for BSLT was found to be 13.59 µg/ml for 48 hrs incubation.

In this study, the use of antibiogram and RAPD to differentiate the different isolates of *S. Typhi* has been demonstrated. IgY was produced against O, H and Vi antigens, this IgY was able to detect even low amount of antigen /S. Typhi in urine by dot blot method. The polyclonal antibody IgY did not showed any cross reactivity with other organisms. We also have shown the applicability of FTIR in diagnosis of *S. Typhi*. Antibacterial activity of methanolic extract of *Swietienia mahagoni* against *S. Typhi* was established.