VI. DISCUSSION

Typhoid fever continues to pose a serious public health hazard in many developing countries (Scunderi et al. 2001). The incidence of typhoid fever has been estimated approximately 17 million cases with 6,00,000 associated deaths occurring annually (Gerald L., Mandell et al. 2002). The distance still remains a serious problem with high mortality rates. Hence a prospective study was performed on current status of *Salmonella typhi* with special references to immunological functions.

1. Incidence of *Salmonella typhi*

The disease is associated not only with non-hygienic conditions, its diffusion is associated with chronic carriers which could remain for a long time as a source of infection and could be particularly dangerous if involved in food handling. The results being varied from nation to nation, the prevalence of *Salmonella typhi* conducted by F.Y. Lin et al., 2000 shows 8.5%, Bhattacharya SS et al. 2003 study shows 12.66%, Shaw MK et al. 2003 study shows 22.7%. U. Madhulika et al. 12.11% and Chowta MN et al. 2005 study shows out of 2842 blood samples 1072 were given positive for *Salmonella typhi* during the period of June 2003 to May 2006 is 37.72%. Whereas in our case the percentage of incidence is little high.

2. Prevalence of typhoid fever

The incidence of typhoid fever was reported by many workers. In the study of Su. Cp. et al. 2004 shows 62.5% in males, 54.1% in females including 62.5% in adults and 37.5% in children. In the findings of WHO, 2004 typhoid fever is more then 100 per one lakh cases per
year. In the present study the prevalence of typhoid fever (Table 01, 02, 03 and Fig. 02) during the period of June 2003 to May 2006 shows 52.61% in males and 47.39% in females. The high rate of incidence was observed more in the age group of 11 to 20, in 39.83% and in males 410.66% also in females 62.33% in the same age group.

3. Identification of *Salmonella typhi*

Detection of the causative agent of typhoid fever mainly includes isolation, cultivation and characterization of *Salmonella typhi* from the blood of suspected typhoid patients. It is still the most reliable diagnostic tool, even though it is not the fastest. Definitive diagnosis of most infection to require the conventional detection of an etiologic agent (Bailey and Scott. 1998) (Table No. 04). Blood samples are generally collected from the suspected typhoid fever patients are inoculated into Brain Heart Infusion Broth and sub cultured on MacConkey gar (MacConkey, 1905) and Wilson Blair Bismuth Sulphite agar (Wilson and Blair, 1931) in the present study. The colonies of *Salmonella typhi* were identified based on the typical and standard, colony characters on the Wilson Blair Bismuth Sulphite agar. Isolated and identified colonies were subjected to a classical gram staining and hanging drop technique to ensure most valuable cellular, morphological information and motility of the suspected pathogens. The isolates were confirmed by Bio-chemical reactions and Antiserums.
4. Antibiogram of *Salmonella typhi*

The twelve antibiotics are used to determine the Antibiogram of *Salmonella typhi* for all the isolates during the period June 2003 to May 2006 (Table No. 05, 06 and 07). The management of the patients with an infection requires the isolation and identification of typhoid pathogens and determination of its susceptibility to antimicrobial agents to detect sensitive drug, invitro susceptibility tests are performed. This method is suitable for organisms that grow rapidly overnight to study the susceptibility pattern as described by Kirby Bauer, *et al.* 1966. This method was widely used and recommended by the National Committee for Clinical Laboratory Standards (NCCLS 2002).

5. Antibiotic Susceptibility Pattern of *Salmonella typhi*

The antibiotic susceptibility pattern has been found to be different in different studies in different countries and this is due to frequent emergence of resistant strains of *Salmonella typhi*. Chloramphenicol introduced in 1948 was remarkably effective for enteric fever worldwide (Bartz, 1972). As a result Chloramphenicol remained the standard treatment for typhoid fever for more than three decades that is up to 1970. Subsequently several reports confirmed the emergence of drug resistance in *Salmonella typhi* (Chogle AR *et al.* 2002). In the study of Ruma Ganguly *et al.* 2001 shows 95% sensitive to Ciproflaxin and Peflaxacin and 67% to Chloramphenicol. Tamilarasu *et al.* 2005 study shows 43% to Ciproflaxin and Chloramphenicol. According to Manchand V. *et al.* 2006 100% sensitive to Cefutoxime, 75% to Ciprofloxacin and 94.5% to Chloramphenicol. In the recent study of V. Lakshmi *et al.* 2006 shows 100% sensitivity to Ceftriaxone and Ofloxacain and 67% to Chloramphenicol whereas our study shows variability with above results (Table No. 08, 09, 10 and Fig. 03).
The sensitivity pattern of *Salmonella typhi* out of 1072 isolates shows higher sensitivity towards Cefuroxime 97.01%, Ceftriaxone 93.65%, Ofloxacin 75.37%, Ciprofloxacin 69.68% and for Chloramphenicol 45.33%. The higher resistance is observed in Nalidixic acid 78.82% followed by Tetracycline 72.66%, Cotrimaxazole 69.77%, Ampicillin 63.71% and Amoxicillin 60.63%.

6. Changing Sensitivity Pattern of *Salmonella typhi*

Sensitivity pattern of *Salmonella typhi* during the period of June 2003 to May 2006 (Table No. 11) shows slight variations. The changing in the sensitivity pattern of Cefuroxime shows 100% and 90.88% respectively for three years. The Ceftriaxone sensitivity pattern is 100%, 81.86% and 100% respectively. This change may be due to mutation and may not due to the plasmids. The decreasing sensitivity pattern is observed in Chloramphenicol from 54.34% to 45.6% and 36.18%. The Amikacin also shows from 72.54% to 45.6% and 25.92%. The antibiotic sensitivity pattern of bacteria can sometimes be characteristic to useful an epidemiological marker for tracing strains.

7. Changing Resistant Pattern of *Salmonella typhi*

The changing in the resistant pattern of *Salmonella typhi* during the period of June 2003 to May 2006 (Table No 12) shows marginal variations. The higher resistance is observed in Nalidixic acid is 81.79%, 81.80% and 72.64% respectively for three years. The higher resistant pattern is observed in Cotrimaxazole i.e. 63.58%, 72.93% followed by Tetracycline 72.54%, 72.5% and 72.93%. The antibiotic resistance is not a new problem but in the recent past the resistance has become worst. All bacteria possess an inherent flexibility.
that enables them to evolve genes that render them resistant to any antibiotic. There is a strong evidence that the major cause of antibiotic resistance is due to inappropriate use of antibiotics by common enteric pathogens has reached alarming levels in India. The rise in resistance may be associated with population patterns particularly crowded living conditions. The availability of antibacterial drugs without prescription may also encourage their indiscriminate use and may help to maintain high resistance levels.

8. MDR Strains

The strains which are resistant to two or more drugs are considered as MDR strains. In 1948, Chloramphenicol became the standard antibiotic for treating typhoid (Woodward et al. 1948), it was not until 1972 Chloramphenicol resistant typhoid fever became a major problem (Mirza SH et al. 1996). Chloramphenicol resistance was associated with plasmids. Outbreaks of infection with MDR strains occurred in all parts of the world. In the study conducted by S.K. Sanghavi et al. 1999 resistant to three or more antibiotic was observed in 60% of isolates. Ushadas et al. 2000 study shows the MDR strains are high as 68.7%. In a recent study of Senthil Kumar B. et al. 2005 the MDR strains were 11.43%. In our study (Table No. 13), the MDR strains for more than two drugs is 3.17%, three drugs 15.48%, four drugs is 5.78%, five drugs 15.48%, six drugs 30.3%, seven drugs 15.2%, eight drugs 6.15%, nine drugs 2.89%. The highest numbers of MDR strains were shows resistance for more than six drugs i.e. 30.3%. Our results were consistent with findings in the study of other authors mentioned above.
9. MIC

The lowest concentration of the drugs which inhibits the growth of organisms is Minimum Inhibitory Concentration (MIC). MIC is determined when a quantitative sensitivity results is required and is the most reliable method. It is the lowest concentration of any drug which will inhibit visible growth of the test organisms after a suitable period of incubation. Normally a serial two fold dilutions of antibiotics are tested under defined conditions against a standard inoculum of the test organisms. Tests may be performed by agar or broth dilutions. The latter may be performed using macro dilutions, micro dilutions or both plus a low content of agar. Micro dilution susceptibility tests (tube method) were performed by carefully following the procedures outlined by the National Committee for Clinical Laboratory Standards (NCCLS, 2000). The criteria of interpreting the MIC results are MIC range, MIC\textsubscript{50} and MIC\textsubscript{90} (Collee et al. 1996) values. The Table No. 26 shows the micro broth dilution protocol to assess MIC of drugs.

In the present study the MIC of all twelve drugs are determined against randomly selected 33 strains along with control strain of *Salmonella typhi* NCTC 786 are shown in Table No.'s 14 to 25. The Table No. 27 shows the MIC range of MIC\textsubscript{50} and MIC\textsubscript{90}.

10. Phage typing, Biotyping and Serotyping

The effective epidemiological surveillance to monitor the present and spread of the disease by *Salmonella typhi* can be distinguished by Phagetype, Serotype and Biotype. In the study of Chhaya Chande *et al.* 2002 shows 77% belonging to E\textsubscript{1} Phagetype and 23% of Phagetype A. Shaw MR *et al.* 2003 study shows Phagetype O was 27.2%, followed by E\textsubscript{1} 21.9%, A 20.4%, Vi 3.3% and UVs 3.6%. The occurrence of Biotype 1 was higher 95.8%
and Biotype II 4.1%. Our study represents randomly selected 100 strains from each year are subjected for Phagetype, Serotype and Biotype shown in Table No. 28, 29 and 30 respectively for three years. For all the three years of duration from June 2003 to May 2006 (Table No. 31) shows Phagetype of E1 is 36.33%, A is 20%, O is 23%, UVS2 is 10%, DegVi is 6.33%, UVS4 is 2.33%, C9 is 0.66% and D is 1.33%; Biotype of Type I is 93.67% and Type II is 6.33% and Serotype of O9, H is 94.67%, O9, H, Vi is 4.67% and O is 0.66%.

11. Determination of CRP

The CRP test has been evaluated by many workers Parikh M. and Singh N., 1995 reported sensitivity and specificity of CRP test as 81.4% and 75.5% respectively. Chan DK and Ho. Ly. 1997 reported sensitivity and specificity values of CRP as 56% and 72%. Anwar SK and Mustaffa S. 2000 showed sensitivity of 60% and specificity of 70%. In a recent study by Ahmed Z et al. 2005 shows the specificity value of 95%. In the present study (Table No. 32) the value of CRP test is sensitivity of 55.03% and specificity of 92.55%. The predictive value of positive test is 92.81%.

12. Buffycoat Smear Study

BCS study is one of the rapid test available for diagnosis of *Salmonella typhi* by non-specific immune mechanism. Chandana A et al. 1998 reported 50% sensitivity. Parikh M. and Singh N. 1995, reported acridine orange stain BCS study sensitivity of 68.5%. Anuradha DE and co-workers 1998, showed the sensitivity of 76.5%. In our study, the sensitivity of BCS is 79.57% (Table No. 33).
13. WIDAL Base Line Titre

For the diagnosis of typhoid fever WIDAL test is the second most widely used after blood culture Frimpong E.H. et al. 2000. In the study of Rasaily R. et al. 1993, showed single WIDAL test titre against ‘O’ antigen is 1:160. Frimpong E.H. et al. 2000 shows the WIDAL baseline titre for anti-O is more or equal to 1:160 and for anti-H is more or equal to 1:320. In the study of Zailani SB et al. 2003 showed baseline titre for Salmonella typhi is 1:160 for ‘O’ and 1:160 for ‘H’. In a recent study conducted by Dutta et al. 2006, showed the cutoff value for anti-O titre is 1:80. Our study, conducted on patients shows antibody titre for O antigen is more in 1:40 and for H antigen 1:80 dilution. (Table no. 34 and Table no. 36). Even our study conducted on community controls shows the antibody titre for O antigen is 1:80 and for H is 1:80 dilution (Table no. 35 and Table no. 37). Hence, the antibiotic titre value of 1:40 for O antigen and 1:80 for H are considered as baseline titre.