III. REVIEW OF LITERATURE

A. Current Status of *Salmonella typhi*. 

1. The Typhoid bacterium

*Salmonella enterica serovar typhi* is a member of the family *Enterobacteriaceae* (Deb et al., 2005). These are gram negative rods measuring about 2–4 μm x 0.6 μm in size. They are non acid fast, non sporing and motile with peritrichous flagella. They are aerobic and facultatively anaerobic. The bacterium is serologically positive for lipopolysaccharide somatic O antigens, protein flagellar H antigen and polysaccharide capsular Vi antigen (Goldstein F.W., 1986). Typhoid fever has received various names such as gastric fever, abdominal typhus, infantile remittent fever, pathogenic fever (Murchison C., 1862), slow nervous fever and putrid malignant fever for typhoid (Huxham, 1782).

2. Pathogenesis

The infectious dose of *Salmonella enterica serovar typhi* in volunteers varies between 1000 and one million organisms (Homick RB, 1970). *Salmonella* organism is able to survive and multiply within the mononuclear phagocytic cells of the lymphoid follicles, liver and spleen (House D, 2001). At a critical point that is probably determined by the number of bacteria, their virulence and the host response, bacteria are released from this sequestered intracellular habitat into the blood stream. The incubation period is usually 7 to 14 days. During the bacteremic phase the organism is widely disseminated. The most common sites of secondary infection are the liver, spleen, bone marrow, gall bladder and payer's patches of the terminal ileum. Gall bladder invasion occurs either directly from the blood or by retrograde spread from the bile. Organisms excreted in the bile either reinvade the intestinal wall or are excreted in the faeces. Counts of bacteria in patients with acute typhoid fever indicate a median concentration of one bacterium per milliliter of blood (about 66% of which
are inside phagocytic cells) and about 10 bacteria per milliliter of bone marrow (Butler 1978, Wain 1998, and Wain 2001).

3. Clinical features

The clinical manifestation and severity of typhoid fever vary with the patient population studied. Most patients who present to hospitals with typhoid fever are children or young adults from 5 to 25 years of age (Osler W. 1912, Stuart BM, 1946 and Huckstep RL, 1962). However, community based studies in areas of endemic diseases indicate that many patients with typhoid, particularly children under 5 years of age, may have a nonspecific illness that is not recognized clinically as typhoid (Lin FY, 2000).

The symptoms in order of frequency is as follows; Fever 100° – 104°F (100%), upper respiratory symptoms like cough and dyspnoea (50%), abdominal discomfort or pain (39%), vomiting (29.26%), diarrhoea (36.5%), headache (34%), mental obundation (20%), malaise and constipation (Sinha 1992).

A recent study showed that 60% patients have continuous fever and rest have intermittent, remittent or irregular fever. Some had fever with chills and rigors. Headache is also a common symptom, especially in children. Constipation is the initial symptom and diarrhoea which comes later on i.e., usually bloody mixed stools (Sinha, 1992).
The patient becomes confused, restless and the abdomen becomes more tumid. There are vague areas of tenderness. Complications usually develop in the 2nd week. The pulse becomes dicrotic or thin, thready and rapid. The temperature remains at a plateau between 101° and 103°F (38.3° to 39.4°C) (Christie AB, 1987).

The patient lapses into the “Typhoid state” which is a dull, disoriented, confused and muttering state also called the “coma vigil”. The pulse becomes thready, respiration becomes rapid and shallow. The face, which is expressionless, also called “the Hippocratic facies” reflects profound toxaemia. This is the stage at which the so called “pea soup diarrhoea” is opted to appear. In the majority of patients, the whole febrile period lasts for about four weeks, but 7 to 17 weeks have also been reported (Christie AB, 1987).

4. Complications

Intestinal Haemorrhage

In the first week, there is evidence of blood in stools. From the middle of second week bleeding of greater severity may occur due to erosion of blood vessels during the ulcerating process of payers patches. When the amount of bleeding is severe, there is usually a fall in temperature, a thready pulse and signs of shock. (Christie AB, 1987 and Gerald T, 1992).

Intestinal Perforation

It occurs in 0.7% to 4% and is responsible for 25% deaths. The classical signs of perforation are uncommon. The perforation is most liable to occur at the lower end of ileum (Bitar R, 1985).
Typhoid Meningitis

It occurs in 1% cases and is more common in children. Clinical picture is similar to that of pyogenic meningitis. CSF yields *Salmonella typhi* on culture. A recent study of typhoid meningitis showed an increase in CSF lymphocyte count (range 6 to 24 cells/cmm) and an increase in protein concentration (range 80 to 125 mg/dl), while glucose concentration remain normal (range 56 to 80 mg/dl). These changes in CSF may resemble viral meningitis from which *Salmonella typhi* meningitis can only be differentiated by culture of CSF (Omprakash Giri, 1993, Varma SN 1991 and Giri O.P. 1992).

Myocarditis

It manifests as cardiogenic shock. The incidence is 46% in an Indian study (Chogle AR 1981)

Genitourinary Complications

One quarter of all typhoid patients excrete *Salmonella typhi* in their urine. This is symptomless bacilluria, especially in the first week. Transient haematuria or proteinurea due to immune complex mediated glomerulonephritis is seen (Chowdhury KL 1988). Patients can present with renal failure or nephritic syndrome. Renal involvement is called “nephrotyphoid”. Acute renal failure can also be secondary to septicaemia and shock. Supurative typhoid pyelonephritis and typhoid cystitis are also known. (Christie AB, 1987).
5. Epidemiology:

Typhoid fever is truly a global disease occurring endemically throughout the developing east sporadically in the west (Kaashif et al. 2002). Many sources of typhoid fever were observed, cross connection of water supply (German and Wolman, 1939), contaminated milk (Wallace and Mackenzie, 1947, Thomas et al., 1948), by handling contaminated fish and salad vegetables (Harmsen, 1954 and Hampesch 1953), frozen egg products, dried coconut (Wilson M and Mackenzie, 1955) and meat (Hemmes, 1951, Nicol, 1956 and Smith AJK, 1966).

Typhoid fever still remains as particularly serious global problem (Scuderi G. et al. 2001). It continues to pose a serious public health hazard in many developing countries (Edelman R. et al. 1986 and Rowe B et al., 1987). The annual incidence in 1995 was estimated to be 16.6 million and approximately 6 lakhs deaths most occurring in Asia (4,40,000) and Africa (1,30,000) (Pang et al. 1995 and WHO 1997).

Recent studies described 214 patients suffering from typhoid fever was endemic. In Bahrain 60-70 cases are being reported annually (Wallace M. 1990, Misra et al. 1997). In a retrospective study during 1988-94 in a non-endemic area of Chicago described the epidemiology of 55 cases of typhoid fever in children & adolescents. Studies in the so called pseudo-endemic zone was the middle east region comprising the persian gulf countries exhibited good number of cases when compared to other parts of the globe. An on going epidemic of typhoid fever in Tajikistan which started in 1996 reported more than 24,000 cases (Tar P.E. 1999) and 8,901 cases were described during the epidemic of 1997 (Mermin et al. 1999).
In South Vietnam, (Am NT et al 1990-1993) reported a total of 3,853 to 9,179 cases per year and isolated *Salmonella typhi* from 369 Vietnamese patients during 1993-96. Four outbreaks described in Vietnam occurred between 1993 & 1997 and 75 *Salmonella typhi* strains were isolated (Connerton et al 2000). A second Asia-pacific symposium on typhoid fever and other *Salmonella typhi* held in Bangkok in 1994. it was reported that, despite its decrease on global scale the incidence of typhoid fever still high in developing countries of Africa & South America which are the endemic zones (Pong et al 1995).

In Egypt incidence of typhoid fever was 0.1-0.2% among school children (Wahdon M.H. 1980 and Glodstein F.W. 1986) of typhoid fever cases from 2,058 in 1978 to 10,342 in 1980. In Santiago, Chile, typhoid fever remained endemic for decoder (Ferreccio et al 1984 and Tanuay et al 1996) reported 3,150 strains of *Salmonella typhi* during the period of 40 years. In Singapore during 1980-89 a steady decline from 5.9 to 1.2 % cases per 1,00,000 population was reported (Yew F.S., 1993). In Honkong, during 1985-97,217 *Salmonella typhi* strain were isolated (Ling J.M,2000 and Tsen, et al 1999) reported 55 *Salmonella typhi* isolated during 1992-96. In Taiwan 71 children were diagnosed with typhoid fever from 1982 to 1995 (Chin C.H., 2000).

A population based surveillance typhoid fever was conducted by Lin FY et al 2000 in three rural communes of Dong Thap province in southern Vietnam (population 28,329) for a 12 month period starting on December 4th 1995, cases of typhoid fever were detected by obtaining blood for culture from residents with fever less than or equal to three days. Among 658 blood cultures, 56(8.5%) were positive for *Salmonella typhi* with an overall incidence of 198 per 10^5 population per year (Lin FY, et al 2000).
In China a study conducted by Hong Hui Yang et al. 2001, the outbreak of typhoid fever began on 1st May 1999 and peaked in the second and third weeks of June 1999. The last case was detected on 24th June 1999. In total, 24 standards met the case definition for typhoid fever during the outbreak, 14 males and 10 females students. (Hong Hui Yang et al. 2001).

In a retrospective study conducted by Su Cp et al., 2004 shows 24 confirmed cases of typhoid fever in Northern Taiwan. There were 15 (62.5%) males and 13 (54.17%) females, including 15 (62.5%) adults (over 16 years in age) and 9 (37.5%) children. Their mean age was 24.7 years (range 9 months to 58 years) (Su Cp et al. 2004).

In the findings of WHO, 2004 a total of 22 eligible studies were identified. Regions with high incidence of typhoid fever (more than 100/100,000 cases/year) include South-central Asia and South-east Asia, Regions of medium incidence (10-100/100,000 cases/year) include the rest of Asia, Africa, Latin America and the Caribbean and Oceania except for Australia and New Zealand, Europe, North America and the rest of the developed world have low incidence of typhoid fever (less than 10/100,000 cases/year). They estimate that typhoid fever caused 21,650,974 illnesses and 2,16,510 deaths during 2000 and that paratyphoid fever caused 5,412,744 illnesses (WHO, 2004).


In Mauritius, the epidemiology of typhoid fever recorded by Issack MI, 2005 between 1997 to 2004 was 25. The infection was likely to have been acquired in Mauritius in only six
cases (24%). Another six cases (24%) occurred in expatriate workers from the Indian subcontinent. Of the thirteen Mauritians (52%) who probably acquired the infection abroad. Eleven had a history of recent travel to India (Issack MI, 2005).

The following studies show the presence of endemic typhoid. In Rourkela, out of 7,866 samples 988 were found positive giving an overall percentage of 12.56 (Bhattacharya S.S. et al 2000). Another study conducted by Ushadas and Bhattacharya S.S. 2000 observed that out of 5,410 blood samples 715 were found positive with a percentage of 13.21 during the period of 10 years (Ushadas and Bhattacharya S.S., 2000).

A study done by Saha M.R. et al, 2003 at Kolkatta shows out of 1,491 samples 338 (22.7%) were positive (Saha M.R. et al, 2003). In Nagpur, 23 isolates of Salmonella typhi give rise to 17.5% (Tankiwale S.S. et al, 2003). In Kolkata, a prospective study done by Lathi Nair et al, 2004 shows 176 positive during the period of eight years (Lathi Nair et al, 2004).

In Pondicherry, a study done by Madhulika U. et al, 2004 shows out of 1,296 suspected patients 157 were positive (12.11%) (Madhulika U. et al, 2004). A prospective study conducted by Vikas Gautam et al, 2002 in Northern India shows out of 6,956 patients 533 were positive (7.66%) (Vikas Gautam et al, 2004). A retrospective study done by Chowta M.N. et al, 2005 at Mangalore, Karnataka, shows a total of 44 positive samples (12.5%) (Chowta M.N. et al, 2005). In a recent study done by Laxmi V. et al 2006 at tertiary care hospital in Hyderabad shows 80 isolates were Salmonella typhi (Laxmi V. et al, 2006).
6. Antibiotic Sensitivity of *S. typhi*

Chloramphenicol formerly known as Chloromycetin discovered in 1947. Since its introduction in 1948 has proven to be remarkably effective for enteric fever world wide (Bartz, 1972). In the pre-antibiotic era, the mortality rate was high as 15%. The introduction of Chloramphenicol in 1948 gradually altered the disease course, decreasing mortality to less than 1% and the duration of the fever from 14 to 28 days to 3 to 5 days. As a result Chloramphenicol remained the standard treatment for typhoid fever for more than three decades i.e., upto 1970’s. Subsequently several reports confirmed the emergence of drug resistance in *Salmonella typhi* (Chogle A.R. et al, 2002).

The antibiotic resistance of *Salmonella typhi* has been changing over a long period of time (Gupta, 1994). Chloramphenicol resistance in *Salmonella typhi* was reported from England in 1950 (Colquhoun et al 1950). Despite the wide spread and often discriminate use of Chloramphenicol in areas of typhoid endemicity, resistance of *Salmonella typhi* to this antibiotic was sporadic prior to 1972. Mexico City witnessed a large out break of typhoid fever due to Chloramphenicol resistant *Salmonella typhi* (Olarte et al, 1973 and Gonzales et al 1973). The majority of *Salmonella typhi* strains isolated during this outbreak were highly resistant to Chloramphenicol both *in vivo* and *in vitro*.

In Varanasi, a study conducted by Gopal Nath, 1999, a total of 140 isolates of bacterium were tested for antimicrobial susceptibility in which Cefuroxime and Ceftriaxone were effective of more than 85% of *Salmonella typhi* tested (Gopal Nath, 1999). In Roorkee, a study of Ruma Ganguly *et al.*, 2001, shows out of 17 isolates of *Salmonella typhi* 95% isolates were sensitive to Ciproflaxacin and Pefloxacin and 67% to Chloramphenicol and 53% to Gentamicin (Ruma Ganguly *et al.*, 2001).

In Rourkela, the study of Bhattacharya S.S. 2000, shows out of 988 isolates Ceftrioxone shows 100% sensitivity followed by Ciproflaxacin 96.9%, Cefotaxime 90.54% and Chloramphenicol 87.46%. (Bhattacharya S.S. *et al.*, 2000). The high degree of sensitivity to Chloramphenicol was also observed. In a correspondence letter by Tankiwale S.S, from Nagpur, 2003 describes their experience of one year study on *Salmonella typhi* to Chloramphenicol was 50% sensitive. They considered the possibility of remergence of sensitivity to Chloramphenicol (Tankiwale S.S., 2003).

The recent Delhi reports shows that, according to Tamilarasu *et al.*, 2005 shows that isolates of *S.typhi* were sensitive to Ciproflaxacin 100% and Chloramphenicol was 43%. According to Manchand V. *et al.*, 2006, 100% sensitivity to Cefotaxime, Cefuroxime and Ciproflaxacin 75%, Chloramphenicol was 94.6% (Tamilarasu *et al.*, 2005 and Manchand V. *et al.*, 2006).

The study of Chowta M.N. *et al.*, 2005 from Mangalore, Karnataka 2005, shows all the 44 isolates were 100% sensitive to Ceftriaxone and Ciproflaxacin sensitivity was 81.9% (Chowta M.N. *et al.*, 2005). In a recent study from Hyderabad conducted by Lakshmi V.
etal, 2006 shows out of 60 isolates 100% sensitivity towards Ceftriaxone, Ceftazidime and Ofloxacin, 67% to Chloramphenicol and 83% to Ciprofloxacin (Lakshmi V. etal, 2006).

7. Multidrug Resistant Salmonella typhi

In 1948 Chloramphenicol became the standard antibiotic for treating typhoid (Woodward etal, 1948). Although resistance emerged within two years after its introduction, it was not until 1972 Chloramphenicol resistant typhoid fever became a major problem (Mirza S.H. etal, 1996). Chloramphenicol resistance was associated with plasmids. These Salmonella enterica serovar typhi strains were also resistant to Sulfonamides, Tetracycline and Streptomycin, but initially Amoxycillin and Trimethoprim-Sulfamethoxazole remained effective alternative drugs. Towards the end of the 1980's and the 1990's, Salmonella enterica serovar typhi developed resistance simultaneously to all the drugs that were then used as first line treatment. Outbreaks of infections with these strains occurred in India (Thresfall EJ etal, 1992 and Shanahan PM etal, 1998), Pakistan (Bhutta ZA, 1999, Shanahan PM etal, 2000), Bangladesh (Hermans PWM, etal, 1996), Vietnam (Hoa NTT. etal, 1998 ), The Middle East (Lancet, 1990) and Africa (Kariuki S. etal, 2000). These multidrug resistant (MDR) strains also carried the plasmids that encoded the resistance genes. Spread results from the clonal dissemination of individual multidrug resistant Salmonella enterica serovar typhi strains or from transfer of the plasmid to multiple Salmonella enterica serovar typhi strains (Thong K.L. etal, 2000,Connerton P. etal 2000 and Mirza S. etal, 2000). Resistance rarely emerges during the course of treatment (Schwalbe RS etal, 1990). Multi drug resistant Salmonella enterica serovar typhi are still common in many areas of Asia, although in some areas strains that are fully susceptible to all first line antibiotics have reemerged(J(Sood S. etal, 1999).
The antibiotic resistance of *Salmonella typhi* has been changing over a long period of time (Gupta, 1994). Chloramphenicol resistance in *Salmonella typhi* was reported from England in 1950 (Colquhoun *et al.*, 1950). Despite the widespread and often indiscriminate use of Chloramphenicol in areas of typhoid endemically, resistance of *Salmonella typhi* to this antibiotic was sporadic prior to 1972. Mexico City witnessed a large outbreak of typhoid fever due to Chloramphenicol resistant *Salmonella typhi* (Olarte *et al.*, 1973 and Gonzales *et al.*, 1973). The majority of *Salmonella typhi* strains isolated during this outbreak were highly resistant to Chloramphenicol both *in vivo* and *in vitro*.

From 1986, antibiotic resistant *Salmonella typhi* have increased all over the world (Gupta 1994). In Mexico, strains of *Salmonella typhi* were found to be multidrug resistant. The resistance pattern included Chloramphenicol, Streptomycin, Sulphonamide, Tetracycline and sometimes even Ampicillin (Olarte *et al.*, 1973). *Salmonella typhi* MDR strain were then isolated in the Indian subcontinent and in South East Asia (Rowe *et al.*, 1987). However the pattern of multidrug resistance changed, becoming more diffuse and including also other antibiotics.

More typical MDR strains of *Salmonella typhi* were resistant to Ampicillin, Chloramphenicol and Cotrimaxazole properly called MDR strains which appeared in Asia since 1980 (Datta *et al.*, 1981, Kohbatta *et al.*, 1983, Fereccio *et al.*, 1984 and Choen *et al.*, 1987). An increase *in vivo* sensitivity to Ampicillin, Chloramphenicol and Cotrimaxazole was described comparing patients treated during 1991 and during 1996 to 1997 (Ranju *et al.*, 1998), while the *in vitro* sensitivity of *Salmonella typhi* to Ciproflaxacin was satisfactory and accounted for 100% of the isolate.
In South Africa only three cases of typhoid fever due to multidrug resistant strains of *Salmonella typhi* have been described in the early 1990. Until 1988, Egypt was relatively free of drug resistant *Salmonella typhi*, occasionally Chloramphenicol resistant strains were isolated (Mikhail *et al*, 1989 and Sippel, *et al*, 1981). In the period of 1991 to 1992, 43% of the 35 *Salmonella typhi* strains isolated in Egypt were found to multidrug resistance to Ampicillin, Chloramphenicol and Cotrimaxazole (Mourand *et al*, 1993 and Wallace *et al*, 1990).

In USA, during 1988-94, multidrug resistant strains to both Ampicillin and Cotrimaxazole were prevalent (Pape *et al*, 1986). Multidrug resistant strains were also prevalent in 293 *Salmonella typhi* strains isolated during 1996 – 1997 (Ackers *et al*, 2000 and Harnett *et al*, 1992). Multidrug pattern of *Salmonella typhi* in Canada during 1992-96 (Rowe *et al*, 1990) reported the isolation of Ampicillin, Chloramphenicol, Cotrimaxazole and Tetracycline resistant *Salmonella typhi* in U.K.

In Varanasi, during the period of 1979 – 1997 in a total of 96 isolates, 79 were MDR strains (82.29%) (Gopal Nath *et al*, 1999). In Pune, during the period of 1991-1998 a total of 556 *Salmonella typhi* isolated. In which the MDR strains i.e. resistance to three or more antibiotics was observed in 60% (Sanghavi S.K. *et al*, 1999). In Orissa a study conducted by Ushadas, 2000 shows out of 715 samples 115 isolates were MDR strains constituting of about 16.1% of total isolates (Ushadas, *et al* 2000).

Chogle A.R. *et al* 2002, in an editorial describes the problem of MDR strains. Antibiotic resistance is progressive evolving from low levels through high levels and once
resistance appears, it likely to decline slowly. Recent reports from centers in India have shown increase in sensitivity of *Salmonella typhi* to Chloramphenicol, Ampicillin and Cotrimaxazole (Chogle A.R. *et al*, 2002).

A prospective study conducted by Lathi Nair *et al*, in 2004 shows that during the period of 1995-2003, the multidrug resistance of *Salmonella typhi* was endemic in Calcutta. In 1989 the MDR strains were 68.7% but since 2002 incidence of MDR strains decreased substantially to 21.05% (Lathi Nair *et al*, 2004). In a recent study of MDR strains, Madhulika U. *et al*, 2004 reported that a total of 157 isolates of *Salmonella typhi* were isolated from blood culture. Of these 131 were Nalidixic acid resistant and 129 Ciprofloxacin sensitive. All isolates were sensitive to Ciproflaxacin. 61 isolates were found multidrug resistant (Madhulika U. *et al*, 2004). MDR isolates of *Salmonella typhi* are on rise and are becoming a challenge for timely and appropriate treatment. In a study of Senthil Kumar B. *et al*, in 2005, among 35 samples 5 isolates were MDR strains. Out of which four MDR were found to have plasmid mediated while in one no plasmid and the chromosome encoded the resistance (Senthil Kumar *et al*, 2005).

8. *Salmonella* Typing

The current status of *Salmonella typhi* strains can be further described by **Biotyping**, **Serotyping** and Phage typing. The effective epidemiological surveillance is needed to monitor the present and spread of the disease.

Chaya Chande *et al*, in 2002 study shows, *Salmonella typhi* was the most common serotype accounting for 51(94%) of isolates. In that 30 isolates were subjected to Phage
typing. Two phage types that is El in 23(77%) and A in 7 (23%) were found to be prevalent in this region. In Nagpur, a predominance of *Salmonella typhi* isolated from 241 patients belonging to phage type El in 186 strains and Biotype I in 225 strains were observed (Agarwal V. *et al*, 1992).

According to the phage typing centre, Department of Microbiology, Lady Hardinge Medical College, New Delhi, during 1992 from various parts of India, reports that a total of 11,391 strains 39.7% were from North India, 37.5% from Central India and 22.8% from South India. The commonest phage type was E1 followed by O and A. This pattern was also seen in the MDR strains of *Salmonella typhi* and certain degraded Vi strains, Untypable Vi strains and Vi negative strains were also multidrug resistant. An important change observed in this study was that a small number of strains belonging to phage types C1, K1, 28, 40, 41 and 42 which were sensitive earlier had developed multidrug resistance. More diversity of phage types was observed in North India as compared to Central and South India (Pillai PK *et al* 1993).

In the study of Ciraj AM *et al* in 1999 shows that out of 226 strains of *S. typhi* isolated over a period of three years, 57.9% of them were MDR strains, majority of the isolates belonging to phage type E (75.7%) and Biotype I (93.8%). Kwai Lin Thong *et al* 2002 found that all strains were susceptible to the antimicrobial agents tested. Among the 81 strains isolated from 1997 to 1999, only four phage types were present: untypable Vi I (UVS1, n=63), untypable Vi (UVS, n= 5), Vi negative (VNS, n=4) and D1(n=9) (Kwai Lin Thong *et al* 2002).
According to Saha MR et al, in 2003 from Kolkatta, reports that out of 338 *Salmonella typhi*, phage type ‘O’ was highest (27.2%), followed by E1 (21.9%) and A (20.4%). The incidence of Phage type C9 and D1 was negligible, 29.9% of strains were untypable with Vi negative strains (25.1%), degraded Vi (3.3%) and UVS (3.6%) strains. The occurrence of Biotype I was higher (95.8%) than Biotype II (4.1%).

B. *SALMONELLA TYPHI WITH SPECIAL REFERENCE TO IMMUNOLOGICAL FUNCTIONS*

Typhoid fever is a global health problem. Its real impact is difficult to estimate because the clinical picture is confused with other febrile infections. Further, the disease is underestimated due to lack of bacteriology laboratories in most clinical settings in developing countries. Incidence of typhoid fever has been estimated as approximately 17 million cases with 6,00,000 associated deaths occurring annually (Edelman R. 1986 National Academy Press in 1986 and Ivanoff BN etal, 1994). Obviously, the disease has a very high social and economic importance (Punjabi N.H. 1998).

Early diagnosis of typhoid fever is critical and depends on alertness of the clinician. However, bacteriological data, unfortunately not available for 24 hours or more occasionally, therapy is delayed or instituted with expensive, potentially toxic or marginally effective antimicrobials. So there is a need for non-culture diagnostic methods to fulfil this problem. In this regard we aimed at correlation of typhoid with CRP test, Buffycoat smear study and Widal Baseline titre.
1. C- reactive protein (CRP)

C-reactive protein (CRP) is an abnormal β-globulin produced during any inflammatory process, bacterial infections, and malignancies and even in tissue destruction by the liver cells as a result of stimulation by interleukin. CRP level takes at least 6-8 hours to rise after the onset of infection and therefore helpful in early diagnosis at the onset of the disease. But after 24 hours CRP value is very helpful and this test also has prognostic value as the levels strongly fall when patient is responding to treatment.

Gerder JS and Polin R, 1998, studied that C-reactive protein test has sensitivity of 47-100% specificity 83-94%, positive accuracy predictive value 62-83% and negative accuracy predictive value 71-99% (Gerder J.S. et al 1998).

Anuradha D.E. and coworkers in 1998 studied 200 cases of septicemia in children. CRP test showed 100% sensitivity and 91.2% specificity. As combination of CRP test and buffycoat smear examination will be very helpful in early diagnosis of childhood septicemia. (Anuradha D.E. et al, 1998).

Krishna BVS etal, in 2000 studied Immunoglobulin-M estimation and C-reactive protein test and blood culture were performed on 57 clinically suspected neonates. IgM level of more than or equal to 20mg/dl was found in 62% and CRP test positive in 68.98% of culture proven sepsis. Of the two tests, CRP had a higher sensitivity while IgM estimation had higher specificity. When the two tests were considered together the sensitivity and specificity further increased (Krishna BVS et al, 2000)
In a study of Anwer S.K., Mustaffa S. 2000 shows C-reactive protein (CRP) and absolute neutrophil count had a sensitivity of over 60% with a specificity of 50%. White blood cell count had a specificity of 93% but a sensitivity of 14%. Also concluded that none of the tests used alone were reliable, but when in combination these tests show a high negative predictive value, the neonate can be discharged early from the hospital, stopping the antibiotics, thereby reducing the cost of treatment and anxiety of the family (Anwer S.K. et al., 2000).

In Malaysia, the study conducted by Choo K.E. et al., 2001 shows a significant positive association between CRP and Widal titres. They investigated the role of serum C-reactive protein (CRP) in the diagnosis of typhoid fever. Upto 227 febrile Malaysian children hospitalized during a twelve month period. The children were, Culture-positive for Salmonella typhi (Group 1: n=108), Culture-negative but with typical clinical features of typhoid fever (Group 2: n=60), or had non-typhoidal illness (Group 3: n= 59). Group 1 children had the highest serum CRP concentrations (Geometric mean range), 43 (12-150) mg/l, 26(8-85) mg/l in Group 2 and 21 (4-110) mg/l in Group 3, P(0.001). In regression analysis, age, patient group and fever duration were independently associated with serum CRP (P<0.05) but gender was not. In Group 1 patients, there was a significant positive association between serum CRP and Widal ‘O’ and ‘H’ agglutination titres. In receiver-operator characteristic (ROC) analysis of serum CRP for Groups 1 and 2 combined. Compared with Group 3, the area under the curve (AUC) was 0.65. These data show that the serum CRP is highest in culture-positive children with enteric fever and reflects the immune response to the infection in the group (Choo K.E. et al., 2001).
The study Ahamed Z. et al, in 2005 shows, CRP was positive in 24/28 (85.7%) of Group-A (Culture proven cases) and 58/72 (80.5%) of Group-B (probable cases) and had a specificity of 95% (Ahamed Z. et al, 2005 March).

2. Buffycoat Smear Study

Faden H.S. in 1976 studied Buffycoat smear from 80 ill patients. In this study 15 patients were blood culture positive. Out of which seven were positive by buffycoat smear examination for the organisms. In this study Buffycoat positivity was 46.66%. This study demonstrates the value of the Buffycoat smear examination in the early diagnosis of bacteremia. It is inexpensive, easy to perform and relatively efficient. In most situations large numbers of bacteria could be seen in each positive smears (Faden HS et al, 1976).

Tak S.K. et al, 1980 studied 30 suspected cases of septicaemia by Buffycoat smear examination for organisms. 15 were blood culture positive, out of which nine cases showed bacteria in Buffycoat layer. Positivity in this study was 60% (Tak SK et al, 1980).

Chandana A. et al, in 1988, studied 50 clinically suspected cases of septicaemia. Blood culture was positive in 48% of cases, among which Gram negative bacilli predominated with 71% of culture positive cases. Buffycoat smears were positive in 12 out of 24 (50%) proven cases (Chandana A et al, 1988).
Parikh M and Singh N. in 1995 studied clinically suspected 254 cases of bacteremia in neonates. 119(47%) were blood culture positive. In this study, Acridine Orange stained Buffycoat Smear (AOS) using fluorescent microscope was used. AOS was positive in 82 of 119 (81.4%) cases showing positive blood culture and negative in 124 of 135 (91.6%) where the blood culture was negative. The specificity and sensitivity of AOS were 91.9% and 68.5% respectively (Parikh M and Singh N. in 1995).

Anuradha D.E. and co-workers in 1998, studied 200 cases of septicaemia in children (age group 0-14 years), out of which 84 were Buffycoat smear positive. Blood culture was positive in 98 cases, among which Buffycoat was positive in 75. Thus sensitivity in this study was 76.5%, specificity 91.2% (Anuradha D.E. et al, 1998).

### 3. Determination of Anti-O and H antibodies

For diagnosing the case of enteric fever, Widal test is the second most widely used test after blood culture (Frimpong E.H. et al, 2000). Agglutination is a classic serologic reaction that results in clumping of a cell suspension by a specific antibody, directed against a specific antigen. Such tests have been widely used for detection of antibodies against various diseases, producing microorganisms in serum for a long time. The Widal agglutinating test, developed by Widal F.M. in 1896 (Widal F.M. et al in 1896) to aid in the diagnosis of typhoid fever, utilizes a suspension of killed *Salmonella typhi* as antigen, to detect typhoid fever in serum from suspected *Salmonella typhi* infected patients who present with febrile illness (Washington J.A. et al, 1991).
The test was based on demonstrating the presence of agglutinin (antibody) in the serum of an infected patient, against the H (flagellar) and O (Somatic) antigens of *Salmonella typhi*, while the definitive diagnosis of typhoid fever depends on the isolation of *Salmonella typhi* from blood, stool, urine or other body fluids (Manson-Balur *et al.*, 1987, Gilman RH *et al.*, 1975 and Gedder A.M. 1974). The role of the Widal test had been to increase the index of suspension for the presence of typhoid fever by demonstrating a positive agglutination during the acute and convalescent period of infection with evidence of a fourfold rise of antibody titre (Sansone *P* *et al.* 1972, Somervile PC *et al.*, 1981 and Anon 1978).

In developed countries, the use of Widal agglutination as a laboratory tool to aid in the diagnosis of typhoid fever during the acute phase of the illness, has largely been abandoned (Washington JA *et al.* 1991, Lateef A *et al.*, 2000). The role of Widal test is controversial, because of the sensitivity, specificity and predictive values of this widely used test vary considerably among geographic areas. But in some countries Papua, New Guinea and Vietnam have found Widal test helpful when it is used with locally determined cutoff points (Widal Baseline titre) (Clegg A. *et al.*, 1994, Parry *et al.*, 1999).

In India the situation is till different, most patients present late to the hospital and require immediate diagnosis and specific treatment and often a single sample has to be relied upon instead of paired serum samples. In that case, high titres of anti-O and anti-H should be considered presumptive for enteric fever. (Jal Punia *et al.*, 2003).
In a study of Rasaily R. et al in 1993, single Widal test was analyzed in patients with bacteriologically confirmed typhoid fever, 116 clinically suggestive but culture negative fever 117, non-typhoidal febrile illness 98 and normal control children 54. Positive Widal test (antibody titre against Salmonella typhi O antigen of 1:160) was recorded in 61.2% of patients with bacteriologically confirmed typhoid fever and in 58.8% with culture negative but clinically suggestive typhoid fever. In contrast, the same titre was observed in 10.2% patients with febrile illnesses of known etiology and in 1.8% of normal children. High specificity and positive predictive value in 1:160 dilution makes the Widal test acceptable as a diagnostic tool.

In the study of Kulkarni et al in 1994, the usefulness of single Widal test in the diagnosis of typhoid fever was investigated. The test was done on 50 normal children, 50 children with non-typhoidal fevers and 30 culture proved typhoid cases. 21(70%) and 9(30%) of thirty typhoid fever cases had ‘O’ and ‘H’ agglutination titre levels of more than or equal to 1:160 respectively as compared to only 3(3%) and 1(1%) among controls. They concluded that based on the above analysis, ‘O’ or ‘H’ titres of 1:160 or more suggestive.

A reappraisal of the Widal test was made for its diagnostic utility in typhoid fever in an endemic area of Central India. The significant basal antibody level in the normal population based on 1200 voluntary per relative blood donors at the cut-off titre of 80 or above was observed in 13.83% for ‘O’ and 8% for ‘H’ antigens of Salmonella typhi respectively. A retrospective study over 138 bacteriologically proven cases of typhoid showed a positivity of 64.49% and 78.26% respectively for ‘O’ and ‘H’ antibodies at the titre of 80 above and 44.2% and at the titre of 160 and above is 63.04%. (Shukla S. et al, 1997).
In Sudan, the study conducted by Shafie S. 1991 shows out of 114 normal individuals *Salmonella typhi* 'O' agglutination were found at a titre of 1:320 in 12 (10.5%) of them. None of these individuals had a history of typhoid fever or vaccination with TAB vaccine.

In Ghana, the study conducted by Frimpong EH. et al, 2000, of the 307 healthy food handlers only 3 (1.0%) had an anti-O titre of more than or equal to 1/160 and 8 (2.6%) an anti-H titre of more than or equal to 1/320 for *Salmonella typhi*, but the majority, 214(69.7%) and 149 (48.5%) had titres of less than 1/120 for O and H agglutinins respectively. Based on these findings, titres of more than or equal to 1/160 and more than or equal to 1/320 for anti-O and anti-H respectively were considered diagnostic for enteric fever in Kumasi, Ghana.

In Nigeria, the study conducted by Zailani SB et al, in 2003. 310 healthy volunteers were admitted into the study and 48 clinically diagnosed and culture positive cases of enteric fever were used as purposeful controls of the 310 healthy volunteers. 274 (97.4%) had reciprocal antibody titre of less than or equal to 80 to H antigen of *Salmonella typhi* on the other hand, in 48 control cases, 43 (89.6%) had reciprocal antibody titre of less than or equal to 160 to O antigen and 38 (78.9%) had reciprocal titre of more than or equal to 160 to H antigen. The sensitivity and specificity of the test were 89.9% and 94.2% for O antibody and 79.2% and 92.3% for H antibody respectively. The baseline titre to *Salmonella typhi* for both O and H antibody is 1:80 where the significant titre for O and H antibodies is 1:160 and above in Nigeria.
The recent study conducted by Dutta S. et al, in 2006 shows the use of rapid and simple diagnostic serological test is useful in India. They collected 6697 patients blood for culture and serological tests and 172 consorting healthy individuals for Widal baseline titres. The cutoff value for Widal test for anti-O titre of 1:80 was optimal with 58% sensitivity and specificity.