Introduction

The term enteric fever should not be viewed as old fashioned. The enteric fevers are severe systemic forms of Salmonellosis. The best studied enteric fever is typhoid fever. The causative organism of typhoid is *Salmonella typhi*, and *Salmonella paratyphi* A, B or C of the paratyphoid fevers. Previously, *S. paratyphi* B was also known as *S. schottmulleri* and *S. paratyphi* C as *S. hirschfeldii*. There are three species of salmonellae and only *Salmonella typhi* and *S. enteridis* species are pathogenic to humans with or without having animal reservoirs. *S. typhi* species has only one serovar i.e., *S. typhi* while more than 2300 serovars including *S. paratyphi* A, B, C etc., belong to *S. enteridis* species. According to the new nomenclature all Salmonellae that cause enteric fever in human are grouped and named *Salmonella enterica* and the previous species names are assigned as serotypes e.g., *S. enterica* serotype Typhi, Paratyphi A, B, C, etc. Salmonella organisms continue to be responsible for significant number of gastrointestinal infections. No country is exempted. Even in the USA in 1978 there were 28,748 isolations of Salmonellae from human sources, including 604 isolates of *Salmonella typhi*.

Pathogenesis

Salmonella organisms penetrate the mucosa of both small and large bowel, coming to lie intracellularly where they proliferate. There is not the same tendency to mucosal damage as occurs with Shigella infections but ulceration of lymphoid follicles may occur. The evolution of typhoid is fascinating. Initially *S. typhi* proliferates in the second part of the Payer’s patches of the lower small intestine from where systemic dissemination occurs, to the liver, spleen, and reticuloendothelial system. For a period varying from 1 to 3 weeks the organism multiplies within these organs. Rupture of infected cell occurs, liberating organisms into the bile and for a second time cause infection of the lymphoid tissue of the small intestine particularly in the ileum. It is this phase of heavy infection that brings the classical bowel pathology of typhoid in its train. Invasion of the mucosa causes the epithelial cells to synthesise and release various proinflammatory cytokines including IL-1, IL-6, IL-8, TNF-β, INF, GM-CSF etc.

Pathology

Huckstep\(^4\) refers to pathology in the Payer’s patches assuming four phases. These phases correspond approximately to the weeks of disease if treatment has not been given.

Phase 1 : Hyperplasia of lymphoid follicles.
Phase 2 : Necrosis of lymphoid follicles during the second week involving both mucosa and submucosa.
Phase 3 : Ulceration in the long axis of the bowel with the possibility of perforation and haemorrhage.
Phase 4 : Healing takes place from the fourth week onward, and unlike tuberculosis of the bowel with its encircling ulcers, does not produce strictures.

Although the ileum is the classical seat of typhoid pathology, lymphoid follicles may be affected in parts of the gastrointestinal tract, such as the jejunum and ascending colon. The ileum usually contains larger and more numerous Payer’s patches than the jejunum, but this is not an invariable finding. It is not generally appreciated that such lymphoid follicles are also found in the large intestine. The number of solitary follicles in large intestine decreases with age. Ulceration
during paratyphoid B infection may involve stomach and large intestine as well.

Egglestone et al. found typhoid perforations as usually being simple and involving the antimesenteric border of the bowel where they appear as punched out holes. In contrast to other types of perforation omental migration to the affected area does not occur.

The reticuloendothelial system, enlargement and congestion of the spleen and mesenteric glands are characteristic finding. The so-called typhoid hepatitis has been described when a liver biopsy may show non-specific reactive hepatitis. The salient features on liver biopsy are focal liver cell necrosis with associated infiltration of mononuclears - typhoid nodules - sinusoidal congestion and dilation, and mononuclear cell infiltration of the portal area. Hepatitis should not be forgotten as one of the complications of typhoid and paratyphoid fever.5

**Laboratory diagnosis**

The laboratory diagnosis of enteric fever is very important mainly because in post-antibiotic era most of the patients are treated empirically by the local medical practitioners and when the fever does not subside, these cases are labelled as pyrexia of unknown origin (PUO) and investigated for various causes of PUO including enteric fever. At this stage the typical signs and symptoms as described above are hardly observed.

The presence of *Salmonella typhi* or *S. paratyphi* is detected either by culture of the organism or by the demonstration of specific antibodies or antigen in the serum or urine. The organism may be cultured from blood, bone marrow, stool or urine.1-2

(i) Culture

In addition to the usual two bottles inoculated with blood, a third bottle containing streptokinase bile salt broth can significantly increase the isolation rate of *S. typhi*. In 210 cases of enteric fever, whole blood conventional bile salt broth yielded the organism in 64% of cases but streptokinase bile salt broth inoculated with blood clot which was minced with scissors yielded a positive result in 92% of cases. Although the conventional wisdom is that *S. typhi* is obtained from blood during the first week of illness more frequently than from the stool, whereas the reverse applies during the second and third weeks of the illness, the clinician should be reminded that the organism can be cultured from blood as late as the fifth week of the disease, and the organism may be cultured from the stool throughout the disease. The organism is less frequently isolated from urine, but it is useful to determine whether a patient does excrete the organism in the urine because this could become a site for chronic carriage. Culture of bone marrow or skin snips taken from rose spots may yield the organism when it cannot be obtained from blood, stool, or urine. The organism can be cultured from the bone marrow in as many as 96% of patients even after antibiotics have already been given. In one group of patients; *S. typhi* was isolated from the blood in 40%, from the stool in 37% and from urine in 7%, but from rose spots in 63% of patients. In a case of paratyphoid fever bone marrow culture yielded the organisms even though antibiotics had been administered. In general the administration to a patient with pyrexia of unknown origin of amoxycillin, ampicillin, or co-trimoxazole inevitably hampers the diagnosis of typhoid fever.1,3,7

The liquid and solid media that are suitable for isolation of *Salmonella typhi* and salmonellosis are several. However, strontium selenite broth is superior to selenite F broth for the isolation of *S. typhi* especially when relatively few typhoid bacilli are present in faeces, for example after antibiotic therapy or if stool specimens have been left for prolonged periods at room temperature; and salmonella - shigella agar has been found to be superior to xylose lysine deoxycholate agar for the isolation of *S. typhi*. Modified bismuth sulphate agar is superior to deoxycholate agar for the growth of Salmonella sp. and is mandatory if the diagnosis of typhoid is very likely, or if a carrier is
being investigated\textsuperscript{1,3,8}.

Automation in clinical microbiology laboratories has been found to be a boon in this direction. The recently introduced Organon-Teknika Bact-Alert automated culture system is one such device. The equipment comprises of non-radioactive highly enriched culture media including a patented resin. This resin can even neutralise the antibiotics in the blood sample, patient might be taking during the sampling time. This facility is also useful because of its speed and computer generated reports and the data analysis. The Salmonella culture can become positive as early as 4 hours after blood sampling. Our laboratory is having this facility, which is only government funded laboratory to have such automation. There are other automated devices like API, vitek etc. All these automated facilities are cost-effective in long run and can provide state-of-the art, prompt, and accurate diagnosis\textsuperscript{9}.

In conclusion, bone marrow is the gold standard for culturing the organism. It can yield positive results even if the patient has started antibiotics. The positivity rate from bone marrow can further be increased to almost 100% if FAN culture medium is used and growth is monitored in automated culture system such as Bact/Alert. Although blood culture is most likely to yield the organism during the first and third week, or septicemic phases of the illness, the clinician is advised to order blood, stool, and urine cultures on one or more occasions to confirm or exclude the diagnosis.

(ii) Serological diagnosis

Antibody detection

The Widal test has long been used as a serological aid in the diagnosis of typhoid fever. Two specimens of serum are required at an interval of 7-10 days and a four-fold rise in the titres of H (flagellar) or O (somatic) agglutinins indicates a strong likelihood of the disease. Previous TAB immunisations may leave residual titres of H agglutinins for years, and a rise in O agglutinins may be more relevant in such patients. However, even in immunised patients it is possible to get a rise only in H agglutinins and not in O agglutinins. The Widal test has the disadvantage that diagnosis is delayed until a second specimen is received\textsuperscript{1,3}. The Widal test can be performed on a single serum, particularly if CIE is not available; elevated titres of O and H agglutinins (e.g. > 1: 320 in and around Delhi) in unvaccinated subjects are strongly suggestive of \textit{S. typhi} infection if the person comes from a non endemic area or is a child less than 10 years old in an endemic area.

Recently a 60 minutes dot enzyme immunoassay for the rapid detection of Salmonella typhi specific IgM and IgG antibodies has been introduced. The test is reported to be 95% sensitive\textsuperscript{10}. The test is now commercialised and available in India.

Antigen detection

However, counter-immunoelectrophoresis (CIE) of a single specimen of serum to detect \textit{S. typhi} O antigen can yield a positive result early in the disease; 96% of 52 sera from typhoid patients were positive with no false positives. In another study in India, on 26 culture proven patients with typhoid, CIE detected 25 out of 26 cases during the early stage-24 positive for \textit{S. typhi} antigen and one for antibody and CIE was also found to be suitable for diagnosis in the chronic or late stages of typhoid fever\textsuperscript{3-4}. Rapid latex agglutination test has also been developed to detect specific antigens in the culture superantants. Its main utility is in rapid identification of species of Salmonella.

\textit{Salmonella typhi} has also a Vi antigen, and antibodies to this antigen can be looked for in a patient’s blood, but it has historically been used to diagnose a chronic carrier of \textit{S. typhi} as described below. \textit{S. typhi} can be subdivided for useful epidemiological purposes by phage typing; there are 80 Vi phage types. Phage typing is required to establish identity of strain between source and patient\textsuperscript{1,3}. 
Diagnosis of typhoid carriers

Carriers of *S. typhi* are either convalescent carriers who excrete the organism for a limited period of time after apparent clinical cure, or chronic carriers in whom persistent excretion of *S. typhi* in stool or urine can be detected a year after clinical illness. Chronic faecal carriers occur more commonly than do chronic urinary ones. The numbers of typhoid bacilli excreted in the stools of these cases may be inordinately large, each gram of faeces usually containing 10 or more viable organisms. The diagnosis of carrier status is established by culturing the organism from the relevant specimen of the suspected person.

Gelatin capsule string test is preferable for detection of chronic faecal carrier. Because excretion of organisms in the faeces of chronic carriers is often intermittent, methods other than faecal cultures have been devised to increase the sensitivity of culture. One such method is to culture the duodenal aspirates in suspected gall bladder carriers. Gilman *et al*\(^1\) have suggested the use of a gelatin capsule containing a nylon string for collecting duodenal specimens. This technique has been found to be highly sensitive and also it can be used for giardia trophozoite demonstration simultaneously.

Vi-antibody tests

Serological tests are used to screen people suspected of being chronic carriers of *Salmonella typhi*. The Vi agglutination test has been used for many years. The Vi test should not be used indiscriminately in screening populations for typhoid carriers, but may have limited usefulness in an attempt to trace suspected carriers. A Vi reactor must be followed-up by bacteriological investigations, but the chance of reactors being a carrier is very small. The demonstration of a carrier among the reactors does not exclude the possibility of another carrier occurring among the non-reactors. The sensitivity of Vi antibody detection is not more than 70%. Moreover the test is not 100% specific too as it has been found false positive in few culture negative cases. There are various tests developed to detect this antigen which include passive haemagglutination, solid phase radioimmunoassay, counter immunoelectro-horesis, and recently the ELISA. All these tests have variable sensitivity and specificity. Vi antigen of Citrobacter coated on red blood cells has also been used to demonstrate the anti-Vi antibodies in blood\(^1,12\).

References