CHAPTER-X
TYPHOID FEVER

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What is Typhoid Fever

- **Typhoid fever**, also known as *typhoid*, is a common worldwide illness, transmitted by the ingestion of food or water contaminated with the feces of an infected person, which contain the bacterium *Salmonella enterica enterica* serovar Typhi. The bacteria then perforate through the intestinal wall and are phagocytized by macrophages. The organism is a Gram-negative short bacillus that is motile due to its peritrichous flagella. The term "enteric fever" is a collective term that refers to typhoid and paratyphoid.
Endemicity of Typhoid Fever
Enteric Fevers

- The syndrome associated with enteric fevers are produced only by a few of the Salmonella
- Salmonella typhi most important
- Salmonella paratyphi A, B, C
Bacteriology – Typhoid fever

- The Genus Salmonella belong to Enterobacteriaceae
- Facultative anaerobe
- Gram negative bacilli
- Distinguished from other bacteria by biochemical and antigen structure
Epidemiology of Typhoid Fever

- This is a highly adapted, human-specific pathogen occurring more frequently in underdeveloped regions of the world where overcrowding and poor sanitation are prevalent.
- According to the best global estimates, there are at least 16 million new cases of typhoid fever each year, with 6,00,000 deaths (Ivanoff, 1995). Between 1 - 5% of patients with acute typhoid infection have been reported to become chronic carriers of the infection, depending on age, sex and treatment regimen. Furthermore this chronic carrier state has also been implicated in causation of carcinoma of the gall bladder.
Antigenic structure of Salmonella

- **Two sets of antigens**
- Detection by serotyping
- 1 *Somatic or O Antigens* contain long chain polysaccharides (LPS) comprises of heat stable polysaccharide commonly.

- 2 *Flagellar or H Antigens* are strongly immunogenic and induces antibody formation rapidly and in high titers following infection or immunization. The flagellar antigen is of a dual nature, occurring in one of the two phases.
Diagnosis of Typhoid Fever (CDC)

- Infection with typhoid or paratyphoid fever results in a very low-grade septicemia. Blood culture is usually positive in only half the cases. Stool culture is not usually positive during the acute phase of the disease. Bone-marrow culture increases the diagnostic yield to about 80% of cases.
he Widal test is an old serologic assay for detecting IgM and IgG antibodies to the O and H antigens of *Salmonella*. The test is unreliable, but is widely used in developing countries because of its low cost. Newer serologic assays are somewhat more sensitive and specific than the Widal test, but are infrequently available.
Diagnosis
Physician Still Plays the Key Role

- Because there is no definitive test for typhoid or paratyphoid fever, the diagnosis often has to be made clinically. The combination of a history of being at risk for infection and a gradual onset of fever that increases in severity over several days should raise suspicion of typhoid or paratyphoid fever.
How we Diagnose Typhoid Fever

- Diagnosis is made by any blood, bone marrow or stool cultures and with the Widal test (demonstration of salmonella antibodies against antigens O-somatic and H-flagellar). In epidemics and less wealthy countries, after excluding malaria, dysentery or pneumonia, a therapeutic trial time with chloramphenicol is generally undertaken while awaiting the results of Widal test and cultures of the blood and stool.
Blood Cultures in Typhoid Fevers

- Bacteremia occurs early in the disease
- Blood Cultures are positive in

1\textsuperscript{st} week in 90%
2\textsuperscript{nd} week in 75%
3\textsuperscript{rd} week in 60%
4\textsuperscript{th} week and later in 25%
Identification of Salmonella

- Sub cultures are done after overnight incubation at 37°C, and subcultures are done on Mac Coney's agar.
- Subcultures are repeated up to 10 days after further incubation.
Salmonella on Mac Conkey's agar
Salmonella on XLD agar
Identifying Enteric Organisms

- Isolates which are Non lactose fermenting
- Motile, Indole positive
- Urease negative
- Ferment Glucose, Mannitol, Maltose
- Donot ferment Lactose, Sucrose
- Typhoid bacilli are anaerogenic
- Some of the Paratyphoid form acid and gas
- Further identification done by slide agglutination tests
Slide agglutination tests

- In slide agglutination tests a known serum and unknown culture isolate is mixed, clumping occurs within few minutes.
- Commercial sera are available for detection of A, B, C₁, C₂, D, and E.
Clot culture

- Clot cultures are more productive in yielding better results in isolation.
- A blood after clotting, the clot is lysed with Streptokinase, but expensive to perform in developing countries.
Bactek and Radiometric based methods are in recent use

- Bactek methods in isolation of Salmonella is a rapid and sensitive method in early diagnosis of Enteric fever.
- Many Microbiology Diagnostic Laboratories are upgrading to Bactek methods.
In 1896, Widal, a professor of pathology and internal medicine at the University of Paris (1911–29), developed a procedure for diagnosing typhoid fever based on the fact that antibodies in the blood of an infected individual cause the bacteria to bind together into clumps (the Widal reaction).
Diagnosis of Enteric Fever

**Widal test**

- Serum agglutinins raise abruptly during the 2\(^{nd}\) or 3\(^{rd}\) week
- The widal test detects antibodies against O and H antigens
- Two serum specimens obtained at intervals of 7 – 10 days to read the raise of antibodies.
- Serial dilutions on unknown sera are tested against the antigens for respective Salmonella
- False positives and False negative limits the utility of the test
- The interpretative criteria when single serum specimens are tested vary
- Cross reactions limits the specificity
Significant Titers helps in Diagnosis

- Following Titers of antibodies against the antigens are significant when single sample is tested:
  - O > 1 in 160
  - H > 1 in 320

  Testing a paired sample for raise of antibodies carries a greater significance.
Widal test
Still a popular test?

- The Widal test (Widal’s agglutination reaction) is routinely practised for the serodiagnosis of typhoid fever by most of the laboratories. Several workers have expressed doubt regarding the reliability of the test. Several factors have contributed to this uncertainty. These include poorly standardised antigens, the sharing of antigenic determinants with other Salmonellae and the effects of immunisation with TAB vaccine. Another major problem relates to the difficulty of interpreting Widal test results in areas where Salmonella typhi is endemic and where the antibody titres of the normal population are often not known.
Limitations of Widal test

Classically, a four-fold rise of antibody in paired sera Widal test is considered diagnostic of typhoid fever. However, paired sera are often difficult to obtain and specific chemotherapy has to be instituted on the basis of a single Widal test. Furthermore, in areas where fever due to infectious causes is a common occurrence the possibility exists that false positive reactions may occur as a result of non-typhoid
Limitation of Widal Test

- The Widal test is time consuming and often times when diagnosis is reached it is too late to start an antibiotic regime.
- In spite of several limitation many Physicians depend on Widal Test.
False Positive and Negative Reactions with WIDAL Test

• The Widal test should be interpreted in the light of baseline titers in a healthy local population. This is especially important when there is a high local prevalence of non-typhoid salmonellosis. The Widal test may be falsely positive in patients who have had previous vaccination or infection with S typhi.

• Widal titers have also been reported in association with the dysgammaglobulinaemia of chronic active hepatitis and other autoimmune diseases.

False negative results may be associated with early treatment, with "hidden organisms" in bone and joints, and with relapses of typhoid fever. Occasionally the infecting strains are poorly immunogenic.
Quality control of Widal tests is important: a laboratory which consistently produces poor results in an external quality control programme should discontinue the test until technical problems are solved. 'False positive results may be due to faulty technique or to poor quality of the antigen suspension. There is conflicting evidence as to the relative importance of somatic and flagellar agglutinin titers for the diagnosis of typhoid fever.
Advances in the Rapid Diagnosis of Typhoid Fever
Typhidot® a test kit that makes use of 50 kD antigen to detect specific IgM and IgG antibodies to *S. typhi* (Ismail et al., 1991). It has undergone full-scale multinational clinical evaluation of its diagnostic value (Lu-Fong et al., 1999; Jackson et al., 1995; Choo et al., 1997). This dot ELISA test offers simplicity, speed, specificity (75%), economy, early diagnosis, sensitivity (95%) and high negative and positive predictive values.
Typhidot is better than Widal Test

- Another variant of Typhidot® is Typhidot-M® and has shown that inactivation of IgG removes competitive binding and allows access of the antigen to the specific IgM when it is present. Evaluation of Typhidot® and Typhidot-M® in clinical settings showed that they performed better than the Widal test and the culture method (Bhutta and Mansurali, 1999).
ELISA Method in Diagnosis of Typhoid Fever

- A new technique of rapid screening for *Salmonella* by dipstick enzyme-linked immuno sorbent assay (ELISA) has been shown to be sensitive, specific, rapid and reproducible for detection of *Salmonella* directly from stool. Stool samples are mixed with buffer *Salmonella* interaction solution. A dipstick is placed into the mixture and incubated at room temperature.

- Results of the tests are available in 20 minutes. Early published results from different studies show a Sensitivity rate of 99% and specificity of 98%. The dipstick kit is very useful and acceptable to both patients and health-care providers because of the following reasons
New Kits being tested in field

- Laboratory confirmation of S. Typhi or S. Paratyphi as the etiologic agent will be essential to distinguish typhoid/paratyphoid from numerous other causes of acute febrile illness. **A rapid diagnostic test (Tubex TF, IDL Biotech, Bromma, Sweden)** can detect typhoid (but not paratyphoid) antibody in patient serum. In field trials, the Tubex TF kit had a sensitivity of 60–78% and a specificity of 58–89%
TUBEX® TF

- TUBEX® TF is a 10 minutes semi-quantitative *in vitro* diagnostic assay for detection of acute typhoid fever. It specifically detects the presence of IgM antibodies to the *S. typhi* O9 lipopolysaccharide antigen. This antigen is highly specific to *S. typhi* and other Salmonella serogroup D bacteria by its extremely rare sugar (a-D-tyvelose). IgM anti-O9 antibodies are normally not
Principles of TUBEX TF

- TUBEX® TF is an inhibition binding assay. In short it detects the presence of anti-O9 IgM antibodies in patient serum by assessing the ability to inhibit a reaction between two colored and antigen/antibody-coated reagents. TUBEX® TF is a semi-quantitative assay, and thus the level of inhibition is proportional to the concentration of anti-O9 antibodies in the sample. The separation is performed in one step by magnetic force; where after the result is read visually and scored against a provided color scale.
RT- PCR in Typhoid

- PCR amplification for the detection of pathogens in biological material is generally considered a rapid and informative diagnostic technique.
- Several experimental methods for PCR methods in progress
- Needs greater validation
Search for Better Methods for Diagnosis of Typhoid Fever

Currently, alternative methods for biological molecular analysis are enzyme immunoassay, surface plasmo resonance, an electrochemical immunoassay. In particular, the use of electrochemical immunoassay has attracted considerable interest for S.typhi determination because of its inherent simplicity, high sensitivity, inexpensive instrumentation, and miniaturization.
Several Emerging Methods in Diagnosis

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Molecular Immunology in Diagnosis of Typhoid Fever

- Recent advances in molecular immunology have led to the identification of potentially more sensitive and specific markers in the blood and urine of patients with typhoid fever and enabled the manufacture of practical and inexpensive kits for their detection.
Molecular Advances in Typhoid Fever
First high-throughput functional analysis of every Salmonella Typhi gene

- The team were able to look at almost all the genes in S. Typhi and showed that it needs only 356 genes for survival: 4162 genes were not essential. Knowing which genes are essential to the survival of pathogens, researchers can seek treatments to target those genes.
Nano Technology in Microbiology

- Nanotechnology is an emerging field that is potentially changing the way we treat and diagnose diseases. The metal-enhanced colloidal gold has not been previously applied to the detection of bacterial cells in real samples.
Nano Technology in Diagnosis of Typhoid Fever

- With the development of nanotechnology, various nanoparticles and Nano quantum dots have been used as labels to enhance the sensitivity of the electrochemical immunoassay technique.