The laboratory diagnosis of enteric fever

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Abstract

The diagnosis of enteric fever currently depends upon the isolation of Salmonella from a patient, most commonly by blood culture. This facility is not available in many areas where the disease is endemic. Serodiagnosis depends upon the 100-year-old Widal test, and other serological diagnostic tools have limitations because of their low sensitivity and/or specificity. The most promising recently published results are from PCR-based amplification of DNA from the blood of enteric fever patients but again this technique is not available where it is most needed. Antigen detection has not been investigated for well over three decades and detecting an immune response specific for typhoid fever has been done only with antibody detection. There is an urgent need for the rational design and evaluation of effective and appropriate diagnostics for enteric fever which must include the emerging threat of S. Paratyphi A.

Key Words: Laboratory diagnosis, typhoid fever, enteric fever, Salmonella Typhi, serology, molecular tests, PCR diagnosis


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Introduction

The World Health Organization (WHO) estimate for annual global incidence of typhoid fever, about 21 million cases [1], is probably an underestimate because of poor diagnostics. Several options exist for diagnosing enteric fever: clinical signs and symptoms; serological markers; bacterial culture; antigen detection; and DNA amplification. None is entirely satisfactory. The clinical diagnosis of typhoid fever is difficult because the manifestations of the disease are diverse [2] and there are many causes of prolonged fever in typhoid endemic regions [3]. Signs such as relative bradychardia or leucopenia may be useful [4] but give a low specificity. The culture of blood, bone marrow and stool are the most reliable diagnostic methods but these are expensive techniques and the infecting organism may be dead on arrival at the hospital if the patient has taken antibiotics before clinical samples can be taken. Serological diagnosis is predominantly by the Felix-Widal test, first standardised in the 1950s, [5]. Although ELISA [6] and immunoblotting [7] suggest possibilities, the commercially available kits for the serodiagnosis of enteric fever have not performed well in large studies [8]. There has been very little commercial interest in developing antigen detection tests.

Diagnosis by culture

Culture of the causative organism remains the most effective diagnostic procedure in suspected enteric fever and where culture is available typhoid fever may account for two thirds of cases of community-acquired septicaemia admitted to hospital [9,10]. Blood has been the mainstay of culture for S. Typhi since 1900. In 1907 Coleman published the first review of blood cultures in typhoid fever [11] and recommended the use of ox bile broth. In 1911 its superior qualities were attributed to the inhibition of the antibacterial activity of fresh blood caused by lysis of blood cells rather than direct enhancement of growth by the bile salts [12].

Reports of the evaluation of different blood culture media suggest that ox bile broth is superior to rich nutrient media, for the isolation of S. Typhi from blood, even when compared to modern blood culture media [13]. While useful for studies on typhoid fever, this media has not found widespread use in general diagnostic laboratories because only bile resistant organisms can be isolated. The addition of saponin to modern blood culture media allows blood to be lysed without inhibiting bile sensitive bacteria; this method was developed in the late 1980s [14], but is not well reported. The isolation of S. Typhi from the bone marrow is considered to be the gold standard method
for the diagnosis of typhoid fever and is reported as more sensitive than blood culture by most, [15,16] but not all, authors [17]. There is a larger number of bacteria found in the bone marrow, tenfold more per volume than in blood, and they may be protected from the presence of systemic antibiotics [18]; however, if enough blood is cultured it may be possible to increase the sensitivity of blood culture to that of bone marrow culture. The use of the blood clot from serum collected for serology is possible but the experience of the authors (JW) is that contamination is problematic and that buffy coat collection provides a higher level of concentration. Stool culture is also an important adjunct for diagnosis; it may be positive when blood culture is negative [19] and it is also important for the monitoring of carriage of S. Typhi after apparent clinical cure, a risk factor for the families of cases [20].

Enrichment media containing selenite are used to isolate S. Typhi from stool because of very large numbers of competing bacteria, especially Escherichia coli [21, 22]. Other sites have been cultured but are not used routinely as diagnostic specimens: culture of the upper gastrointestinal tract using a duodenal string can be valuable but the technique is poorly tolerated by young children [23]. S. Typhi can be grown from rose spots [24] but rose spots are often difficult to see and may only be present in 4% of cases [25]. S. Typhi can also be grown from urine but may be associated with urinary tract infection rather than typhoid fever [26].

**Antibody detection tests (serology)**

**The Widal**

The Widal agglutination test, suggested by Widal more than one hundred years ago for the diagnosis of typhoid fever [27,28], detects serum antibodies to the O=9,12 somatic, the H=d flagellar, and the “Vi” capsular antigens of S. Typhi. The interpretation of the Widal test remains problematic to this day, with a great number of articles reporting different cut-offs [29] and the test has lost some popularity in recent years as antigenic determinants of both typhoid and non-typhoid Salmonella organisms are now characterised.

In many places, instead of the standard Widal test, a quantitative slide agglutination test [30] is used but this should always be interpreted with reference to clinical data. According to the original papers, a rise in titre over time or a single high test result is diagnostically significant and this is supported by modern studies using ELISA. False negative results may occur if the blood is collected too early in the disease; therefore, negative results do not rule out typhoid fever [31] and may be best used as a baseline for subsequent comparative titrations [31]. False positive results may be associated with a past history of immunization for typhoid fever, cross-reacting antibodies [6], or a whole host of infections and conditions.

The detection of Vi antibodies can be used for detection of carriers during specific investigations [32,33] but is not routinely performed in most diagnostic laboratories and the use of a Vi Widal reagent using tube agglutination has not been well reported.

**Haemagglutination (HA) Tests**

Many researchers have evaluated the usefulness of HA tests in different countries. In a study from India, the anti LPS HA test showed a sensitivity of 60% and specificity of 98.2%. The positive predictive value and negative predictive value were 66.7% and 96.7% respectively. In the same study, the haemagglutination inhibition test targeted Salmonella antigens and was found useful for helping the early detection of S. Typhi in culture [34]. In another study, a Reverse Passive Haemagglutination Test (RPHA) was designed for the detection of S. Typhi antigen. The test was found to be 70% sensitive and 92% specific for acute typhoid fever diagnosis [35]. These studies indicate that the passive HA test is comparable with the Widal test and may be a useful alternative to the Widal test for the serological diagnosis of typhoid fever in busy microbiology laboratories in areas in which the disease is endemic [36].

**Countercurrent Immunoelectrophoresis (CIE)**

This test is based on electrophoresis and the visualization of the precipitin band of antigen-antibody complexes that form. The sensitivity is similar to that of the Widal test and the procedure may be quicker if tests are batched (about one hour for a gel), but bands are often difficult to see, the cost is higher than that of the Widal, and some studies conclude that CIE has a low sensitivity with Vi antigen. A panel of antigens (somatic (O), flagellar (H) and capsular polysaccharide (Vi) antigens of Salmonella typhi) is recommended for rapid diagnosis of typhoid fever [37].

**Rapid tests**

The clinical application of a dot blot test to detect IgG (88% sensitivity and specificituy) and IgM (12.1% sensitivity and 97% specificity) against the flagellar antigen from Salmonella enterica serovar Typhi has been performed in Peruvian and Colombian patients.
with 100% specificity [38]. The TyphiDot is a DOT enzyme immunoassay (Typhidot and Typhidot-Mt; Malaysian Biodiagnostic Research SDN BHD, Kuala Lumpur, Malaysia) that detects either IgM or IgG antibodies against a specific antigen on the outer membrane protein of serotype Typhi. This test is designed for the rapid diagnosis of typhoid fever even in areas with limited resources. Some studies showed that the Typhidot and Typhidot-Mt gave superior results to the Widal test in their diagnostic sensitivity and specificity [39] but a large study of over 6,000 cases of fever found no advantage over the Widal test and reported problems with the quality control of different batches of kits [8]. A dipstick assay that was developed for use in developing countries has been trialed and, although superior to the Widal, again lacks either sensitivity or specificity (sensitivity 77%, specificity 95%) (58% and 98.1%) [40]. The advantages of the dipstick assay are that the result can be obtained on the same day, allowing a prompt treatment; only a small volume of serum is needed; no special laboratory equipment is needed to perform the assay; and the reagents remain stable when stored at room temperature [41].

TUBEX (IDL Biotech, Sollentuna, Sweden) is a semiquantitative test that uses polystyrene particle agglutination to detect IgM antibodies to the O9 antigen. TUBEX detects antibodies from a patient's serum by the ability of these antibodies to inhibit the binding between an indicator antibody-bound particle and a magnetic antigen-bound particle. The TUBEX test uses a colorimetric reaction which may be masked in hemolyzed samples, and false positives may occur in persons with recent S. Enteritidis infection [42]. Of the currently available commercial kits trialed in developing countries, TUBEX seems to perform best [43] but none may actually be better than the 100-year-old Widal test [8]. It is a very rare study that reports specificity and sensitivity above 95%. This means that at least 1 out of 20 patients is misdiagnosed; surely in the 21st century this is unacceptable for such a widespread disease as enteric fever.

**Antigen detection tests**

There is clearly a demand for a simple diagnostic test for enteric fever. An ideal test is reliable, simple, and affordable for the countries where the need is greatest. Many of the affected countries are poor, and some places do not have electricity. Perhaps the under-explored antigen detection, rather than antibody detection, could provide such a test.

**Protein antigens and Vi**

S. Typhi antigen can be detected in the urine of some typhoid patients by co-agglutination [30] and ELISA [44,45] but specificity varies from 25-90%. Testing of urine during the first week of fever onset for Vi antigen using the ELISA with a monoclonal Vi capture antibody detects most patients with typhoid fever [46].

**DNA detection tests**

Given the problems associated with the diagnosis of enteric fever by both culture and serological techniques, many authors have explored the use of PCR for detecting specific DNA sequence in clinical specimens from patients. The food industry has used PCR technology for several decades and guidelines are published for quantitative detection of Salmonella in food by PCR [47]. While there are several very good studies looking at the detection of S. Typhi DNA from clinical material, medical science is not at the same level of standardisation as the food industry, and there is very little published data on the detection of S. Paratyphi A. Below is a survey of recent literature on DNA amplification techniques but direct comparison is impossible at this stage. What is needed is a laboratory comparison of the different target DNA sequences used for diagnosis so that the most appropriate can be recommended and hopefully used.

Studies using single [48] or nested [49] PCR primers for fliC of S. Typhi have reported good results from PCR. Using samples from 40 clinically suspected cases of typhoid fever, 20/20 culture positive and 12/20 culture negative cases were positive by PCR in Delhi, India [50]. Using single primer PCR in South Sulawesi, Indonesia, 46/73 blood samples collected from patients with clinically suspected typhoid fever were positive compared to 13.7% positive by blood culture [51]. In Varnassi, India, nested PCR (53/57 positive) was again better than blood culture (17/53 positive) on specimens from 63 clinical typhoid fever cases and 25 healthy controls [52].

A large, well-designed study in Indonesia investigated 131 patients with a clinical diagnosis of typhoid fever and found diagnosis by PCR of DNA from blood (84.5%) and urine (69.3%) to be slightly more sensitive than blood culture (61.8%) [53]. A study from Nepal on specimens from 71 children with suspected typhoid fever reports 82.7% positivity for PCR from blood and urine, both much higher than blood culture (26.9%). In Pakistan, 55 cases of
suspected typhoid fever and a control group of 20 healthy persons (PCR versus blood culture) gave 58.2% versus 14.5% positivity, respectively [54]. The authors of the study conclude that the sensitivity of PCR as compared with that of blood culture was significantly better [54]. Again in Pakistan, a multiplex PCR targeting five different genes for differential diagnosis of typhoidal pathogens has been developed for use directly on clinical blood samples. Of 42 multiplex PCR-positive blood samples, 35 were positive for S. Typhi and two for S. Paratyphi A. Interestingly, five patients were found to have mixed infection [55].

Despite this wealth of data, we have reviewed only some of the more recent articles; PCR has not become an established method for diagnosis typhoid fever.

Conclusions

The diagnosis of enteric fever depends upon techniques, blood culture and PCR, which are not available in the very areas where the disease is most common. The Widal test and other serological diagnostic tools have limitations because of their low sensitivity and/or specificity.

There is an urgent need for the rational design and evaluation of effective and appropriate diagnostics for enteric fever. These must include the emerging threat of S. Paratyphi A.

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References


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