

## BRIEF REVIEW

# NEW ADVANCES IN THE DIAGNOSIS OF TYPHOID AND DETECTION OF TYPHOID CARRIERS

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**For effective management of typhoid, diagnosis of the disease must be done with speed and accuracy. Development of such a test would require antigens that are specific for typhoid diagnosis. Attempts at finding the specific antigen have been carried out throughout the years. The finding of such an antigen can lead to carrier detection as well. Candidate antigens have been used in the development of antigen or antibody detection tests with variation in sensitivity and specificity. Further characterization and understanding of the candidate antigens combined with use of innovative technologies will allow for the ideal test for typhoid and typhoid carriers to be within reach.**

*Key words : New advances, typhoid diagnosis, typhoid carriers, typhoid antigen*

### Introduction

Typhoid fever remains a public health problem in most developing countries with an estimated incidence of 540 per 100,000 population (1). Since typhoid may mimic the symptoms of other fevers including dengue, malaria, hepatitis and scrub typhus, in typhoid endemic regions, results obtained from the laboratory are important in confirming the clinical diagnosis of typhoid and will contribute to the effective management and treatment of typhoid cases. The conventional diagnosis for typhoid include the culture method and antibody detection tests. Although variations to the conventional techniques have improved both tests, the search for better and improved tests still prevails.

The continued high incidence of typhoid is due to the dissemination of the disease via typhoid carriers (2). Hence there is an urgent need to increase the chance of detecting the carriers so as to decrease the risk that they pose to the communities. In urban areas where sewage disposal is lacking or inadequate, public water supplies are contaminated and typhoid fever is common. The contamination

of food by food handlers who are carriers, forms the second commonest route of infection. Since spread of the disease is via fecal-oral route, attempts at breaking the transmission cycle would contribute toward the effective control of the disease. Control of typhoid outbreaks include screening of food and water samples to trace the source of the aetiologic agent. The availability of diagnostic tests that are rapid, sensitive, specific, simple to perform and cost effective to detect for the pathogen in contaminated food, water and healthy human carriers, would provide an effective tool in controlling and preventing typhoid.

In the quest of developing an accurate test for typhoid fever, there is a need to discover antigens specific for typhoid diagnosis. The finding of such an antigen can lead to diagnostics for carrier detection as well. Studies have been done throughout the years to search for the specific antigen. Attempts at utilizing the antigens toward the development of a suitable diagnostic test have been reported with variation in sensitivity and specificity. Our increasing understanding of the candidate antigens and the use of innovative

technologies for laboratory procedures are addressed in this review. It is the intention of this review to elucidate the use and effectiveness of the various diagnostic tests available for the diagnosis of typhoid fever and for the detection of typhoid carriers.

### **Conventional methods of typhoid diagnosis**

Current diagnosis for typhoid is still via the method of culture and antibody detection by means of the Widal test. Isolation of *Salmonella typhi* has remained as the gold standard, with culture the bone marrow aspirate or a combination of specimens from blood, stool or urine. However, it is well recognised that facilities for culture are not readily available or are limited in many areas. Although the culture method may show specificity, it however lacks sensitivity and speed. If positive, culture produces results within 2-7 days, but culture negative typhoid is well recognised (3). Culture is also less sensitive for diagnosis of infection among children compared to adults (4,5,6). The culture method despite its shortcomings in speed and sensitivity is still useful for antibiotic sensitivity testing.

The value of the Widal test, which uses the bacterial agglutination technique for the diagnosis of typhoid and paratyphoid fevers, has been assessed by several investigators. In endemic areas where culture facilities are lacking or limited, the Widal test remains among the few tests available to differentiate enteric infection from other illnesses due to bacteria, viruses or animal parasites (7). However, it is also recognised that agglutination tests have serious shortcomings (8). Discrepancies in results between laboratories or even within the same laboratory have been reported especially when preparations of the antigens had come from different sources (9,10). There is also evidence that among patients who have been proven as typhoid cases, detection of antibody against the O and H antigens has not been demonstrated by the Widal test (11). On the other hand, antibodies against *Salmonella typhi* have been detected among nontyphoid *Salmonella* infections (12) and sometimes even in diseases not caused by *Salmonella* (13). For meaningful interpretation of the test, demonstration of a 4 fold rise in antibody titers between acute and convalescent sera, at least 10-14 days later, is essential. In the clinical settings, it is common practice to make an interpretation based on a single serum specimen which may not reflect the diagnostic value of the test. More often even when paired sera are obtained, a decrease in titer is commonly

observed when comparing the convalescent titer to the acute titer. This could be due to the fact that most patients attended the hospital during the convalescent phase, after initial pretreatment by the general practitioners failed.

When interpreting the Widal test it is of utmost importance that the test be interpreted against the background normal titer of the population in question. It is not uncommon to find what is considered positive in a non-endemic area may be considered normal in an endemic area. The interpretation of the tests may also vary among the endemic areas.

Despite problems of accurate diagnosis associated with the Widal test, studies have shown that the test may be useful among febrile paediatric patients in endemic areas (14).

### **Advances in typhoid diagnosis**

An ideal diagnostic test for typhoid and typhoid carriers should be rapid, specific as well as sensitive. The development of a rapid and specific test combined with sensitive diagnosis would provide for prompt, effective management and control of typhoid fever. The existing conventional tests lack speed, sensitivity and specificity. To overcome the limitations of the existing tests, new specific antigens and new diagnostic techniques have been employed. Some of the antigenic candidates include outer membrane proteins (15), lipopolysaccharides (16) and heat shock proteins (17). The need for an alternative, low cost test for typhoid has also spurred the development of other serological assays including counterimmunoelectrophoresis (18), ELISA (19), RIA (20) and the haemagglutination assay (21). Coagglutination tests have also been used for the detection of antigens in urine and serum (22,23), and DNA probes have been suggested for the detection of *S.typhi* in blood (24). However, none of the tests have so far obtained widespread acceptance in microbiological laboratories. Since typhoid fever is common in developing and underdeveloped countries, the race toward development of the ultimate ideal test still continues. This is because the development of such a test will have a huge economic significance as well as impact on public health management for all endemic countries in the region.

Outer membrane proteins (OMP) due to their location have been primed as important candidates

to elicit host immune response (16,25). Although several possible antigenic candidates have been elucidated from studies on the OMPs, only the 50 kDa protein has undergone a full scale multinational clinical trial in order to evaluate its diagnostic value (26,27,28,29). The 50 kD outer membrane protein was determined to be antigenic as well as specific for *Salmonella typhi* since it only reacted immunologically with typhoid sera (30). Further evaluation of the antigen using the dot enzyme immunosorbent assay (EIA) method revealed that the 50 kD antigen could detect for the presence of specific IgM and IgG in sera from patients with acute typhoid (31,32,33,34).

Evaluation of the tests in clinical settings, showed that the dot EIA test (*TYPHIDOT*) offers simplicity, speed (1-3 hours), specificity (75%), economy, early diagnosis, sensitivity (95%) and with high negative and positive predictive values (31). When interpreting the test, detection of IgM would reveal acute typhoid (early phase of infection) while detection of both IgG and IgM would also suggest acute typhoid (middle-phase of infection). In highly endemic areas where the rate of typhoid transmission is high, the detection of specific IgG will increase. Since IgG could persist for more than 2 years after typhoid infection (34) the detection of specific IgG could not differentiate between acute and convalescent cases. Furthermore, cases of false positive results due to previous infection may also occur. On the other hand, IgG positive may also occur in the event of current re-infection. In cases of re-infection, there will be a secondary immune response with a significant “boosting” effect of IgG over IgM such that the latter could not be detected hence “masking the effect” of IgM (35). One possible strategy to resolve the problems mentioned is to detect for the presence of IgM by making sure that its presence is “unmasked” (35,36). To increase diagnostic accuracy in these situations, a modification to the original *TYPHIDOT* test was done by inactivating total IgG in the serum sample. Studies with the modified test called *TYPHIDOT-M* have shown that inactivation of IgG would remove competitive binding and allow accessibility of the antigen to the specific IgM, when present. The detection of specific IgM (within 3 hours) would suggest acute typhoid infection. Evaluations on the *TYPHIDOT* and *TYPHIDOT - M* tests in clinical settings showed that both tests performed better than the Widal test and even the culture method (35,36).

In the laboratory diagnosis for typhoid fever,

the method used as the gold standard should approach 100% in terms of its sensitivity, specificity, positive and negative predictive values. Evaluation studies have shown that *TYPHIDOT-M* was superior to the culture method (35,36). Although culture remained as the gold standard, it could not compete with *TYPHIDOT-M* in terms of sensitivity (>93%), negative predictive value as well as speed (35,36). *TYPHIDOT-M* could also be used to replace the Widal test when used in conjunction with the culture method for the rapid and accurate diagnosis of typhoid fever. The high negative predictive value of the test suggested the usefulness of *TYPHIDOT-M* in a highly endemic area.

### Finding the typhoid carrier

The human population is a reservoir as well as natural host for several enteric pathogens. These asymptomatic carriers represent an important reservoir that helps to perpetuate the disease and is responsible for the outbreaks of enteric diseases including typhoid fever. Approximately, 2-5% of typhoid cases become chronic biliary carriers and hence perpetuate the endemicity of the disease (37). The chances of becoming a carrier increases with age and is evidently greater among women (38). The detection of these carriers thus is an important aspect of disease control. The current gold standard to detect for carriers is by means of stool culture. This is not only tedious and costly, it also has a low sensitivity (39). Multiple bacteriological examination of stools are also necessary to make a reliable diagnosis due to intermittent or light fecal excretors among carriers. There have been studies on carriers that showed positive fecal culture only after 196 negative culture results (40). Hence there is a need to have an alternative serological carrier detection system that is not only specific, sensitive and cost-effective but is also easy to use in the field.

When developing a serodiagnostic test it is important to determine the antibody that would be an indicator for carrier diagnosis. Studies with Vi antigens (41) have shown that IgG is the primary indicator for carriers and IgM does not play a role. IgA can be found in both the acute and carrier state. When further tested, IgA and secretory IgA were found most frequently in the sera of dysentery and typhoid carriers. No secretory IgA was detected among vaccinated individuals (42). When quantifying the content of immunoglobulins in different forms of typhoid fever, the carrier state showed high IgA and IgG content which began as

early as the acute period (43). Other studies have also shown that IgA among carriers seemed to be elevated 2.4 times compared to non carriers (but with a previous typhoid history) while IgM is only elevated among acute typhoid cases (44). IgG was found to be high among typhoid and typhoid carriers. The high IgA content among carriers may reflect prolonged immunological stimulation since IgA when formed does not last long in the body (in cases of acute typhoid). It has been suggested that the continuous presence of IgA among typhoid carriers may be due to *S.typhi* being the primary occupant of the biliary system during chronic infection (41). Hence IgA detection among healthy individuals may also indicate typhoid carrier state.

Among the antigens used in the serological screening for carriers (45,46) none equaled the Vi antigen in terms of widespread acceptance as an indicator of typhoid carriers (47). Various techniques have been used in the development of a diagnostic test which uses the Vi antigen from *Citrobacter freundii*. Passive hemagglutination assay has been used and was found to show high specificity and sensitivity especially when used in highly endemic areas (47,48). However, the sera needed to be preabsorbed with sheep erythrocytes before being used and this may not be convenient for screening of large populations. Counterimmunoelectrophoresis has also been used and had high sensitivity and specificity when compared to the hemagglutination test (49). Converting the test to the ELISA format has also been tried without much success since the Vi antigen showed poor binding to the microtiter plates (50) except when tyraminated (41). The ELISA test using tyraminated Vi antigen was recommended for carrier diagnosis with IgG as the indicator at equal to or greater than the cut-off titer of 1:200. The test has a sensitivity of 86% and a specificity of 95% (41).

## Conclusion

The anti-Vi test is still used for the lack of a better test for carrier diagnosis. The ideal carrier detection test should be easily used and interpreted in the field rather than in the laboratory to allow for immediate diagnosis. The development of a test using the immunochromatography or dipstick method may be more useful for convenient carrier detection in the field. Development of multi-test for simultaneous typhoid and typhoid carrier diagnosis will be able to have a greater impact on the control and management of typhoid fever.

Multi-tests developed for the detection of the organism in environmental samples would also enhance typhoid control since it would allow for tracing of the source of contamination.

While carrier detection is important for public health, reports have also shown a close relationship between biliary disease and chronic carriers (51). Recent developments have shown that the risk of gallbladder carcinoma has increased among typhoid carriers (52,53,54). Hence a test to detect for typhoid carriers that is cheap, sensitive, specific and user friendly for field work would promote not only effective management but also reduce gallbladder carcinoma and dysfunction.

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